

Wiadomości Lekarskie Medical Advances



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Memory of
dr Władysław
Biegański

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ABSTRACT

Marie Skłodowska-Curie Symposia on Cancer Research and Care (MSCS-CRC) promote collaborations between cancer researchers and care providers in the United States, Canada and Central and Eastern European Countries (CEEC), to accelerate the development of new cancer therapies, advance early detection and prevention, increase cancer awareness, and improve cancer care and the quality of life of patients and their families. The third edition of MSCS-CRC, held at Roswell Park Comprehensive Cancer Center, Buffalo, NY, in September 2023, brought together 137 participants from 20 academic institutions in the US, Poland, Ukraine, Lithuania, Croatia and Hungary, together with 16 biotech and pharma entities. The key areas of collaborative opportunity identified during the meeting are a) creating of a database of available collaborative projects in the areas of early-phase clinical trials, preclinical development, and identification of early biomarkers; b) promoting awareness of cancer risks and efforts at cancer prevention; c) laboratory and clinical training; and d) sharing experience in cost-effective delivery of cancer care and improving the quality of life of cancer patients and their families. Examples of ongoing international collaborations in the above areas were discussed. Participation of the representatives of the Warsaw-based Medical Research Agency, National Cancer Institute (NCI) of the United States, National Cancer Research Institutes of Poland and Lithuania, New York State Empire State Development, Ministry of Health of Ukraine and Translational Research Cancer Center Consortium of 13 cancer centers from the US and Canada, facilitated the discussion of available governmental and non-governmental funding initiatives in the above areas.

KEY WORDS: cancer prevention, cancer care, clinical trials, education, training, research

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HISTORY, NEEDS AND GOALS

Marie Skłodowska-Curie (1867-1934) was a Polish expatriate and a pioneering scientist with groundbreaking contributions to the understanding and treatment of cancer. She was born in Warsaw, Poland, Nov 7, 1867. Studies and started her teaching and early laboratory work in Warsaw (1889-1891), before moving to Paris in 1891. Dr. Skłodowska-Curie was the first woman to earn Nobel Prize and the only person to win Nobel Prizes in 2 different disciplines (Physics, 1903; for work on radiation) and Chemistry (1911; discovery of Radium and Polonium). She trained 4 other Nobel Prize winners, including her daughter, Irène Joliot-Curie, and Frédéric Joliot-Curie, and founded Radium Institute of Paris (1914) and Warsaw Radium Institute (1932), which became the current Maria Skłodowska-Curie National Research Institute of Oncology (MSCI) in Warsaw, Poland.

When staying in Buffalo to meet her childhood friend, Dr. Francis Fronczak, a health commissioner of the Erie county in June 1921, Dr. Skłodowska-Curie visited New York State Pathological Laboratory of the University of Buffalo (now Roswell Park Comprehensive Cancer Center) first Cancer Center in the US, established in 1898 by a cancer surgeon, Dr. Roswell Park, known for his effectiveness in promoting private and governmental action in the area of health research (Wyrobek, 2022). Dr. Skłodowska-Curie's visit provided an important stimulus for early work on radiation biology and cancer treatment at Roswell, but also helped her to mobilize funds to support her work in Paris, and the Warsaw Radium institute. 1g of radium presented to Dr. Skłodowska-Curie by President Harding allowed her to advance her work on radioactivity applications in cancer and

other diseases. Her work fundamentally changed how we detect, treat and monitor the progress and effect of cancer treatments.

That success in leveraging the complementary expertise and resources available in the US and in Europe provided us with inspiration for the Marie Skłodowska-Curie Symposia on Cancer Research and Care (MSCS-CRC) initiative, started in May 2019, in conjunction the Buffalo-held 60 Million Congress/Global Polonia Summit, which brought together cancer researchers, educators and cancer care providers from the United States and Poland. After two years of Covid-related gap, the second edition of the MSCS-CRC, was held as a hybrid meeting in September 2022. The well attended meeting (112 participants) provided a platform for presentations by respected leaders of healthcare systems from Poland, Ukraine, Hungary, Czech Republic, Croatia, Canada and the United States. Basic and clinical oncology research, medical education and training, prevention, genetics, biomarkers, as well as oncology patient care were main topics discussed at the Meeting. The conference was very successful and showed opportunities for further international collaborations, to build stronger integrated research and healthcare systems in Eastern and Central Europe partnering with American institutions. The third edition was held as fully in-person meeting at the newly renovated Gaylord Cary Conference center of the Roswell Park Comprehensive Cancer Center on September 2023, brought together 137 participants from 20 academic institutions in US, Poland, Ukraine, Lithuania, Croatia and Hungary, and representatives of the Polish-American, Ukrainian-American, Romanian-American, Hungarian-American, Czech-American

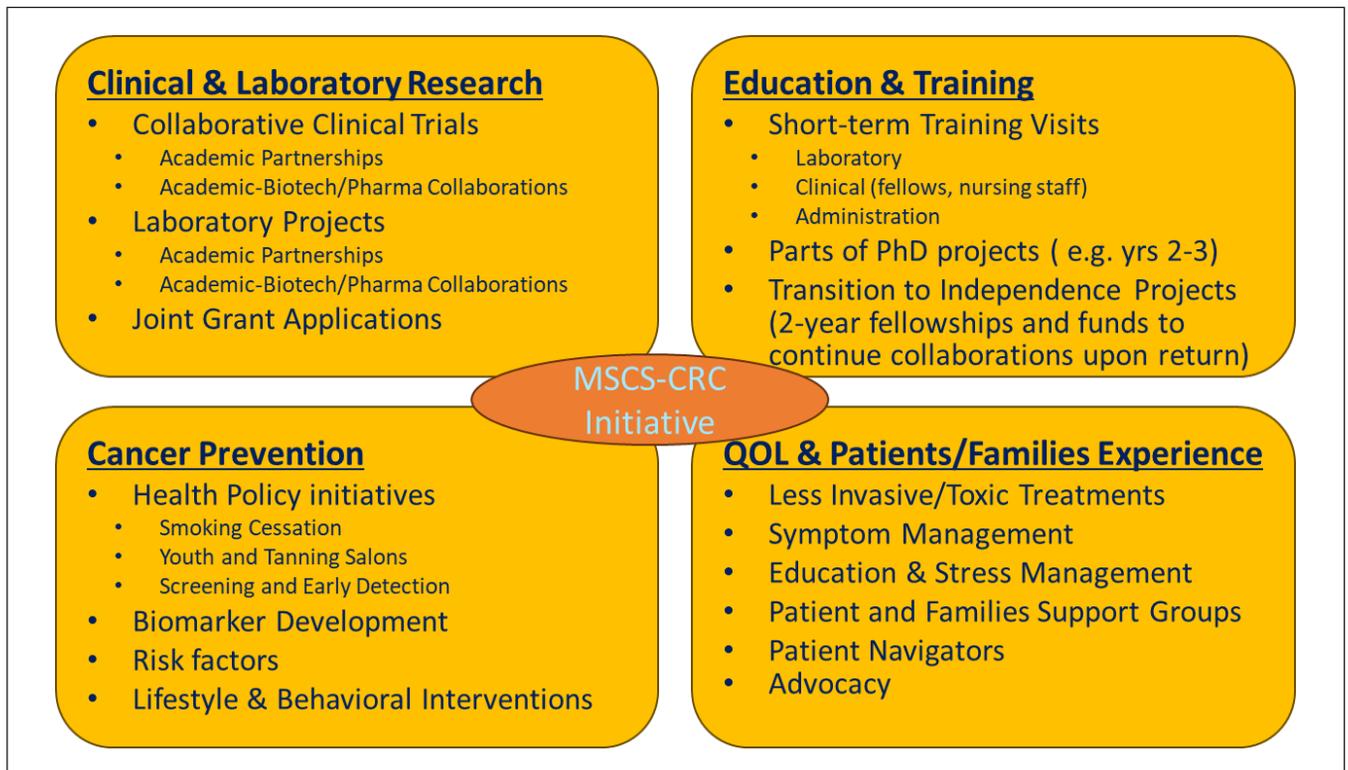


Fig. 1. Key areas of programmatic emphasis discussed during MSC-2023.

and Serbian-American research communities, as well as representatives from 16 biotech and pharma entities from the US and Poland (<https://www.roswellpark.org/msc-symposium>).

The overarching goal of the MSCS-CRC initiative is to promote collaborations between cancer researchers and care providers, biotech, pharma and policymakers from the US, Canada and Central and Eastern European Countries (CEEC). The 2023 Symposium focused on the needs and arising opportunities for research collaborations in the areas of joint laboratory and clinical research projects in cancer prevention, early detection and treatment, new health policy initiatives, education and training, as well as improvement of the quality of life of cancer patients and their families (Figure 1).

The development of the database connecting the parties interested in preclinical and clinical collaborations will be coordinated by the National Institute of Oncology, Warsaw Poland, and Roswell Park Comprehensive Cancer Center, Buffalo, NY.

Social events provided additional opportunities for networking and small group discussions (Figure 2).

Promoting collaborative clinical and laboratory research: Lower saturation with clinical trials performed in CEE Countries, combined with a strong clinical trials base and oversight (European Medicines Agency, a partner for the FDA and <https://www.biomapas.com/running-clinical-trials-in-eastern-europe/>), documented by multiple FDA audits, and approximately 50% lower costs of clinical trials, compared to the of the US, represents a unique opportunity to the US-CEE teams to accelerate early phase testing of new therapies developed in academic or commercial settings. Lower costs of the laboratory research in CEEC and high experience with many unique areas, such as novel drug delivery methods and mathematical approaches to biologic questions and image analysis, combined with the shortage of qualified research personnel in many parts of the US further creates a unique opportunity for the development of preclinical and translational projects involving partners from both continents, with opportunities for high impact publications, rapid clinical testing and commercialization. Dissemination of results from early phase trials of low-cost therapies with potential to enhance the effects of immunotherapy or radiotherapy (stress management, beta blockers, NSAIDs) and improve overall cancer outcomes conducted at Roswell and other institution, and their expansion into multi-center pivotal studies, was an example discussed in several presentations.

Education and training initiatives: Several of the speakers discussed their experience from training international clinicians and laboratory scientists in their labs. The challenges discussed included current difficulties in converting such short-term visits into career-building

programs and long-term collaborations, as a result of lack of suitable funding mechanisms.

Health policy initiatives, cancer awareness, prevention, early detection, and biomarkers represents another area of immediate opportunity to reduce cancer incidence, improve early diagnosis and initiation of treatment, with the overall goal to enhance survivorship and quality of life. Two of such areas discussed at MSC-2023 were smoking and vaping cessation and early-life UV exposure, relevant to, respectively, lung- and skin cancers.

Empowering patients and caregivers: Patient-centered care, survivorship, quality of life and advocacy. Empower patients and caregivers with knowledge, resources, and support systems to navigate the challenges of cancer, and actively involve them in discussions regarding research priorities and patient-centered care. Discuss new challenges of care treatment at the time of war and other disasters.

SCIENTIFIC SESSIONS OF MSC-2023: HIGHLIGHTS

The 2023 MSC Symposium was divided into an Opening Session and eight subject matter sessions, each involving individual presentations and panel discussions:

The Opening Session: The meeting was opened by **Pawel Kalinski, MD, PhD**, Senior Vice President for Team Science at Roswell, representing **Renier Brentjens, MD, PhD**, the Deputy Director of Roswell Park, and **Ms. Camille Brandon***, representing the General Pulaski Association of Western NYS. Welcome remarks were delivered by Elected Officials including **Karen Utz, JD**; WNY Regional Director, Empire State Development representing NYS **Governor Kathy Hochul** and **Jesse Prieto** representing **Congressman Nick Langworthy**. Other elected officials included NYS **Senator Timothy Kennedy**; Erie County Legislator **Hon. April Baskin**; Deputy Mayor of Buffalo, **Hon. Callie Johnson** and City of Buffalo Councilman **Hon. Bryan Bollman**. Roswell Park Board of Directors was represented by **Elyse NeMoyer**, herself a two-time cancer survivor, and **R. Buford Sears**, Senior Vice President for the Buffalo-based M&T Bank. The Session was concluded by Dr. Kalinski provided a historical perspective linking Dr. Skłodowska-Curie, Roswell Park and American Polonia, as well as the cultural and history of economic and political links between United States, Canada and Countries of Central and Eastern Europe, and an overview of the goals of the Symposium and its agenda.

Session 1: Ongoing Collaborations within the MSC-CRC Initiative was chaired by **Iwona Ługowska, MD, PhD** (NIO, Warsaw, Poland) and **Michael Nishimura,**

PhD (Loyola University, Chicago, Maywood, Illinois, USA). The goal of the session was to use the examples of the effective collaborations developed in the course of the past interaction between the members of the MCS-CRC Initiative to highlight the existing needs and immediate collaborative opportunities, encountered problems and ways of their elimination.

Michael Nishimura, PhD (Loyola University, Chicago, Maywood, Illinois, USA) provided an overview of his recent experience training medical scientists from Poland, Ukraine and Lithuania and transferring inexpensive CAR and TCR T Cell Technology to collaborating groups in Europe. He discussed the encountered roadblocks and experience overcoming them, as well as plan for extension of his collaborations to Ukraine.

Iwona Ługowska, MD, PhD (Maria Skłodowska-Curie National Research Institute of Oncology and the National Institute of Oncology, Warsaw, Poland) discussed her experience in establishing effective international collaborations in clinical research, especially early-phase trials with pharma partners. She highlighted the role of MSCI (originally founded in 1932 as the Radium Institute by Maria Skłodowska-Curie) as a comprehensive cancer center, encompassing both research and clinical aspects of oncology, as well as implementing high-quality patient care standards throughout Poland. In addition to the Warsaw HUB, MSCI has branches in Gliwice and Krakow, allowing it to provide care to patients for the 25% of cancer patients in Poland. This includes over 130,000 in-patients, 680,000 outpatient consultations and diagnosing 50,000 new patients annually. Currently, MSCI manages over 650 clinical trials (including 80 early-phase trials). Its Early Phase Clinical Trial Department, Center for Excellence in Precision Oncology, and the newly developed CAR-T Facility provide top-notch expertise and partnering opportunities for domestic and foreign collaborators. The Cancer Prevention Centre involved in many international projects, e.g. the Nordic-European Initiative on Colorectal Cancer, European Polyp Surveillance Trial and Era-NET Transcan). MSCI is a leader of the National Cancer Strategy, the National Oncology Network, and the National Coordinating Center for population screening and primary cancer prevention and runs the National Cancer Registry. Additional partnerships involved ESMO, ASCO, NCI, EORTC, NCCN, ASH, EURACAN, EuroBloodNet, EndoERN, Institute Curie, University of Leeds, MD Anderson's Sister Institution Network, EHA, WSN, EuMelaReg, EBMT, and different patient advocacy groups. Recently, MSCI signed Memorandums of Understanding (MOUs) with the National Comprehensive Cancer Network (NCCN) and the U.S. National Cancer Institute, leading to the implementation of NCCN guidelines in Poland and the

establishment of educational programs such as the School of Cancer Epidemiology.

Participation in research and infrastructure projects funded by the European Commission adds another dimension to MSCl's capabilities, propelling the development of innovative therapies and positioning Poland as a hub for cutting-edge technologies. With ongoing collaborative research projects, including JA JANE, JA, CRANE, CCI4EU, 4UNCAN, PCM4EU, PRIME-ROSE, CAN. Heal, MELCAYA, STRONG-AYA, ECHOS, and EUonQLQ, MSCl remains at the forefront of oncology research.

As a result of the 2nd MSC Symposium held in Buffalo in September 2022, MSCl has established collaborations with groups in Warsaw and Poznan, in Poland to transfer their gene therapy technology and establish a country-wide clinical gene therapy program with the goal of helping cancer patients in Poland. So far, three scientists from other centers have visited and trained at MSCl.

Leszek Kotula, PhD (Upstate Medical University, Syracuse, New York, USA) discussed the new collaboration with the NIO initiated at the MSC-2022 Symposium, which resulted in the establishment of a collaboration between Upstate Medical and NIO. Is focused on prostate cancer biomarker, ABI1 in advanced and therapy-resistant prostate cancer cases. ABI1 was identified as a biomarker by the group of Dr. Kotula at Upstate Medical University, while suitable patient cohorts for this study will be led by the NIO. The collaborating groups have already laid the groundwork for securing joint funding to support this initiative.

Pawel Kalinski, MD, PhD (Roswell Park) discussed his collaborative experience involving multiple academic centers, pharma and biotech partners in manipulating the induction and effector phase of immunity in tumor microenvironments (TME) and developing chemokine-modulating (CKM) regimen involving TLR3 ligand, rintatolimod and IFN α 2b (Muthuswamy et al., 2012; Muthuswamy et al., 2016; Muthuswamy et al., 2017; Muthuswamy et al., 2015; Obermajer et al., 2018; Theodoraki et al., 2018), which led to two recently-published positive clinical trials in ovarian cancer (local application of CKM) (Orr et al., 2022; Suarez Mora et al., 2022) and breast cancer (Gandhi et al., 2023), which demonstrated the ability of CKM to exert local effectiveness in advanced multi-focal disease even after its systemic application, due to its synergy with activated NF κ B, which cancers selectively activate local TME to promote their growth, vascularization and local immune suppression, but which can be utilized by the CKM to assure its local intratumoral selectivity even after its systemic application, converting immuno-resistant "cold tumors" into immuno-sensitive "hot"

tumors, without the need for local or targeted delivery. Dr. Kalinski discussed ongoing and upcoming trials of CKM in combination with chemotherapy, dendritic cell (DC) vaccines and PD1 blockade in melanoma, prostate, breast, ovarian and colorectal cancers, as well as in infectious diseases, and the opportunities for collaborations with partners in Poland and other CEE Countries, to address these questions in larger cohorts of patients. The final part of his talk discussed the logistic, fiscal and regulatory experience of the office of Scientific Programs Management, which he established at Roswell with importing foreign-produced drugs and obtaining FDA and local regulatory approval for their use as Investigational New Drugs in the clinical trials in the US.

Elizabeth Repasky, PhD (Roswell Park, Buffalo, NY, USA) discussed the role of psychologic stress and beta-adrenergic signaling pathway in promoting tumor growth, suppressing antitumor immunity and undermining the effectiveness of immunotherapy and radiation therapy in mice and cancer patients (Bucsek et al., 2017; Chen et al., 2020a; Eng et al., 2015; Gandhi et al., 2020; Gandhi et al., 2021; Kokolus et al., 2013; Kokolus et al., 2018; Mohammadpour et al., 2021). The recently published results of a phase I study in melanoma patients (Gandhi et al., 2021) indicate that beta blockers, such as propranolol can be used as safe low-cost enhancers of checkpoint blockade and potentially other forms of cancer. Ter retrospective analyses performed with **Anurag Singh, MD, PhD** at Roswell, provide rationale for similar intervention to enhance the effectiveness of cancer radiotherapy (Chen et al., 2020b; Farrugia et al., 2021a; Ma et al., 2021). Opportunities for partnering with CEEC collaborators were discussed, to advance the use of propranolol in wide groups of cancer patients.

Session 2: Opportunities for New Collaborations in Translational & Clinical Research was chaired by **Marcin Kortylewski, PhD** (City of Hope, Duarte, California, USA) and **Piotr Rutkowski, MD, PhD** (Medical Research Agency and Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland). The session on translational and clinical research in cancer presented a number of new opportunities for collaborations. The speakers focused on the potential of new approaches to cancer prevention, diagnostics and novel treatments based on cancer vaccination or CAR-based NK and T cell immunotherapies. The session provided an opportunity for networking and collaboration between scientists from different disciplines and institutions. As highlighted in the discussion by **Dr. Kortylewski**, effective cancer treatments need to combine strategies disrupting tolerogenic TME with expanding and armor-

ing immune effector cells against local suppression to ensure the long-term antitumor responses.

Gyorgy Paragh, MD, PhD (Roswell Park, Buffalo, NY, USA) discussed opportunities for prevention and treatment of squamous skin carcinoma by targeting early clonal mutations. He discussed their role in cancer progression and the feasibility of inducing immunity against these patient-specific mutational events as a pathway to skin cancer prevention. The theme of cancer vaccines was continued by **Wei-Zen Wei, PhD** (Karmanos Cancer Institute, Detroit, Michigan, USA) who presented her experience in targeting Her2 in the immunotherapy of breast cancer. Her presentation targeting new checkpoint molecules to enhance the clinical effectiveness of HER2 vaccination.

Sandro Matosevic, PhD (Purdue University, West Lafayette, Indiana, USA), discussed the role of cell-based immunotherapies utilizing natural killer (NK) cells in the treatment of cancer, and the existing challenges and opportunities for the broad deployment of these therapies to patients worldwide. NK cells, immune cells with innate ability to recognize and eliminate cancer cells, are powerful immune effectors, representing a widely applicable allogeneic immune cell. The dysfunction of endogenous NK cell function in the TME of solid tumors involves immune, epigenetic and metabolic mechanisms, both intrinsic and extrinsic to cancer and immunosuppressive immune cells, which dampens NK cell activation. These involve downregulation of NK activating receptors and upregulation of inhibitory receptors, as well as additional suppression involving other immune effector cells. The approaches to restore NK cell immuno-metabolic effector responses have involved pharmacological and genetic tools to regulate NK cell activation, their anti-tumor function, metabolism and enhancing their persistence in the TME by cytokine modulation. Dr. Matosevic discussed opportunities for using NK cells in immunotherapy, their novel genetic engineering approaches and therapeutic modulation strategies, with the ultimate goal to effectively and durably target the currently incurable cancers in patients in the US, Europe and beyond.

The last speaker in the session, **Renier Brentjens, MD, PhD** (Roswell Park, Buffalo, NY, USA), discussed Roswell Park's current expanding efforts to transfer his experience from the particularly successful CAR-T cell program in hematologic malignancies to solid cancers by the identification of the new antigenic targets relevant to highly heterogeneous solid tumors and the development of the next generation "armored" CAR T cells for solid tumor malignancies, able to overcome tumor heterogeneity and the highly immunosuppressive nature of the solid tumors TME.

Session 3: Opportunities for New Collaborations in Cancer Biomarkers & Prevention was chaired by

Leszek Kotula, PhD (Upstate Medical University, Syracuse, New York, USA) and **Sandro Matosevic, PhD** (City of Hope, Duarte, California, USA)

Petar Ozretić, PhD (Ruđer Bošković Institute and Croatian Association for Cancer Research (HDIR), Zagreb, Croatia) gave an overview of preclinical cancer studies carried out at the Institute, the largest basic research institution in Croatia. The work covers molecular mechanistic and functional *in vitro* analyses as well as molecular profiling of clinical samples, to better understand the signaling pathways involved in cancer development, such as the Hedgehog-Gli signaling pathway.

Daniel J. Powell, Jr, PhD (University of Pennsylvania, Philadelphia, Pennsylvania, USA; President of the Translational Research Cancer Centers Consortium, USA; <https://www.trccc.org/executive-board>) discussed the biomarkers of tumor-specific TILs which identify effector-like stem T cells with particularly high anti-tumor potential in adoptive T cell therapies. He emphasized the heterogeneity exists in intratumoral T cells in solid cancers and the role of CD137 as a reliable biomarker for identifying tumor-specific TILs. Biomarker based enrichment of TIL enhances their activity in adoptive T cell therapy of common epithelial cancers, permits TCR isolation, and deep interrogation of natural endogenous T cell immunity to cancer. Isolated CD137+ TILs contain T cells with stem-like properties which are still heterogeneous and include a non-canonical CD45RO+ Tcf-1+ stem-like TILs subset representing one of the earliest effector subsets in the TME. These unique cells maintain self-renewal capacity without a transcriptional commitment to exhaustion. Dr. Powell discussed the properties of this subset and other subsets of endogenous T cells relevant to controlling cancer progression in context of clinical trials of the TIL-based and transgenic-TCR-based adoptive T cell therapies (ACT).

Elżbieta Sarnowska, MD, PhD (Maria Skłodowska-Curie National Research Institute of Oncology, Warsaw, Poland) discussed the character of T cell responses in kidney cancer and opportunities for their therapeutic manipulation. One of the hallmarks of cancer is immune system evasion. Proliferating tumor cells produce distinct suppressive cytokines and other molecules to inhibit the immune system attack. Prompted by the 2007 Polly Matzinger mouse study showing advantage of therapeutic use of CD4+ T cells, Dr. Sarnowska, demonstrated that, CD4+ TILs are predominant in clear cell renal cell carcinoma (ccRCC) TME compared to CD8+ TILs. CD4+ effector T cell become exhausted upon chronic activation and display PD-L1 and IDO1 as suppressive molecules, in addition to proinflammatory cytokine. Interestingly, the CD4/CD8 TILs ratio in primary tumors showed promise as a prognostic marker,

raising the need for larger prospective studies, which will benefit from collaborations.

Vita Pašukonienė, PhD (Vilnius Gediminas Technical University and National Cancer Institute, Vilnius, Lithuania) discussed her work development and use of a gene expression-based immune subtyping tool (IMSUBTOOL) for solid cancers, which utilizes a gene expression pattern maps as an algorithm for immune subtyping of high-grade serous ovarian carcinoma. The results allow to identify tumors with a better prognosis using only a few genes related to immune mechanisms and the TME, rather than the whole tumor genome. The group is interested in new collaborations to further develop immune subtyping tools to assess treatment efficacy in large prospective clinical trials and retrospective studies of immunotherapy.

Andrew Hyland, PhD (Roswell Park, Buffalo, NY, USA), presented his international collaborative efforts in reducing incidence of cigarette smoking, which is the leading cause of cancer death in the US, Poland, and many other countries. He discussed new initiatives to reduce cigarette smoking, including tobacco “quitlines”, as highly cost-effective health interventions. The Polish National Quitline and the New York State Smokers Quitline have been in operation for 20+ years and there are opportunities to apply successfully practices across borders to improve program success metrics. Five tangible areas of collaboration include: 1) strategies to increase marketing reach of tobacco cessation services; 2) improved protocols for telephonic delivery of tobacco treatment; 3) improved protocols for remote access to stop smoking medications; 4) increased training for health professions to treat tobacco dependence; and 5) enhanced digital services including evidenced-based text messaging programs tailored to different languages/populations.

Session 4: Opportunities for New Collaborations in Early Phase Clinical Trials was chaired by **Elizabeth Repasky, PhD** (Roswell Park, Buffalo, NY, USA) and **Daniel Powell, PhD** (University of Pennsylvania, Philadelphia, PA, USA).

The session was started by **Kęstutis Sužiedėlis, PhD** (National Cancer Institute, Vilnius, Lithuania) presenting his collaborative projects in search for more efficient modalities of cancer radiotherapy. **Brian Czerniecki, MD, PhD** (Moffitt Cancer Center, Tampa, Florida, USA) and **Gary K Koski, PhD** (Kent State University, Kent, Ohio, USA) discussed the role of DCs in cancer immunotherapy and the possibility of using the paradigms of natural infections to enhance their potency. They showed that human DCs loaded with synthetic tumor antigen peptides and activated with purified bacterial cell wall components *ex vivo*, and injected intratumorally, prime tumor-specific T cells, but also

promote local infiltration of multiple immune effector populations and create conditions for tumor elimination. Intratumoral delivery of such DCs combined with tumor-directed monoclonal antibodies resulted in dramatic tumor regressions in preclinical mouse models. A 12-subject phase I neoadjuvant clinical trial for locally advanced HER2 breast disease combining intratumoral DCs plus anti-HER2 monoclonal antibody drugs likewise showed dramatic regressions in most patients with no side effects, suggesting this approach should be further developed. Several multi-institutional clinical trials of different DC variants involving Moffitt Cancer Center and Roswell Park are currently accruing patients and can be extended to additional interested partners. In each trial, all autologous DC vaccines are produced at a single site (depending on the expertise with each specific DC type, either at Moffitt or at Roswell), to assure uniformity of the product. In case of patients from the other institution, the vaccine is prepared from the shipped leukapheresis material of each patient at the DC production institution (which holds the IND) and shipped back in a frozen form to the treating institution.

Iwona Ługowska, MD, PhD (National Institute of Oncology, Warsaw, Poland) discussed the impressive collaborative expertise of the Early Phase Clinical Trial Department and partnering opportunities for domestic and foreign collaborators resulting from the newly developed CAR-T Facility of the NIO’s Center for Excellence in Precision Oncology with. She also discussed the collaborative experience of the NIO’s Cancer Prevention Centre, already involved in many international projects, such as the Nordic-European Initiative on Colorectal Cancer, European Polyp Surveillance Trial and Era-NET Transcan). MSCI is a leader of the National Cancer Strategy, the National Oncology Network, and the National Coordinating Center for population screening and primary cancer prevention and runs the National Cancer Registry.

Waldemar Priebe, PhD (MD Anderson, Houston, Texas, USA) shared his experience connecting academic and biotech researchers in the US and in Poland in order to accelerate the development of novel cancer therapies. He discussed the feasibility of conducting clinical trials of the US-developed drugs in Poland and the value of seeking public - private partnerships in this area.

Session 5: Sources of Governmental Funding for International Collaborations was chaired by **Piotr Rutkowski, MD, PhD, and Mark Parascandola, PhD, MPH** (National Cancer Institute, Bethesda, Maryland, USA), and focused on the availability of state, federal and local governmental sources of funding available to support international collaborations in the area of cancer.

Piotr Rutkowski, MD, PhD, discussed the role of the Medical Research Agency (MRA) of Poland as the vehicle for development of new technologies in clinical medicine. MRA supports innovations in healthcare system, financing medical and health science research, and providing funding for non-commercial clinical trials. It supports the implementation of the National Oncology Strategy and responds to unmet medical needs of the society. Within the area of oncology, so far MRA funded 54 non-commercial clinical trials and 10 commercial clinical trial projects (at the level of 250,000,000 USD or 1 billion PLN) in the areas of non-commercial clinical trials and laboratory projects, R&D, head-to-head comparisons, rare diseases, targeted/personalized medicine, digital technologies, new pharmaceutical forms. MRA funds allowed for the establishment of 7 Oncology Clinical Trial Support Centers across Poland, contributing to creation of a structured and efficient organizational structure for cancer centers. They helped to increase the availability of clinical trials in oncology by increasing their overall number, standardization of operational processes related to their conduct, introduction of quality and other support systems, supporting adequate infrastructure and increased patient awareness of the importance of oncologic clinical trials. In 2024, new requests for proposals (RFP) to support innovative diagnostic and therapeutical solutions, targeted and personalized cancer therapies, digital technologies in healthcare, non-commercial clinical trials in cancer and a separate RFP for Regional Centers of Digital Medicine with Biobanking, will provide a strong background for multi-country collaborations including the CEE Region and the US.

Mark Parascandola, PhD, MPH (National Cancer Institute, Bethesda, Maryland, USA), provided overview of the role of the U.S. National Cancer Institute in supporting international collaborations to advance cancer research, through extramural grant funding, intramural research collaborations, and bilateral agreements. Over 10% of NCI extramural awards in 2022 involved a foreign collaborator. The NCI's Center for Global Health, established in 2011, supports global cancer research and training in low- and middle-income countries with a particular focus on the use of technology, implementation science, and health disparities (<https://www.cancer.gov/about-nci/organization/cgh/about>). The National Institutes of Health has also utilized bilateral funding agreements to support collaborative international cancer research, such as the Academy of Finland (AKA) –National Institutes of Health (NIH) Partnership Program (<https://grants.nih.gov/grants/guide/notice-files/NOT-OD-21-021.html>). In the ensuing discussion, Dr. Rutkowski, Dr. Kalinski and Dr. Mark Parandascola, PhD, from

the NCI (Bethesda, Maryland) discussed opportunities for potential bilateral collaboration between MRA and NCI in these areas.

Karen Utz, JD (Empire State Development; <https://esd.ny.gov/regions/western-new-york>), discussed the ESD's efforts at promoting healthcare & biotech Innovations in New York State. Ms. Utz discussed the individual tools aimed at promoting domestic and foreign investment in New York state, ranging from state grants, through matching funds to tax credits.

Session 6: Education & Training- Needs and Opportunities was chaired by **Adam Kisailus, PhD** (Roswell Park, Buffalo, NY, USA) and **Brian Czerniecki, MD, PhD** (Moffitt Cancer Center, Tampa, Florida, USA). The session addressed opportunities for undergraduate, graduate and post-graduate training available to domestic and international students. As highlighted by **Dr. Kisailus**, comprehensive cancer centers represent fertile grounds for education and training future cancer scientists and oncologists in an innovative, collaborative, and interdisciplinary environment. He highlighted the informal and formal educational opportunities to engage students as early as middle school in understanding cancer science, immerse college and health professional students in research experiences, to train graduate students through partnership with the University at Buffalo in the Roswell Park Graduate training programs in cancer sciences and prepare post-doctoral and graduate medical students for careers in academic research and oncology medicine. Several current faculty members at Roswell Park are alumni of these different programs and attest to their education and training at Roswell Park as confirming their pursuit of cancer careers and providing the preparation necessary to reach their career goals. The above sentiment was mirrored in the presentation of **Dr. Czerniecki**, who focused on his experience training of laboratory and clinical scientists at Moffitt. In the following discussion, **Drs. Suźiedelis and Kalinski** argued for the need of programs allowing foreign trainees to complete fellowship (or a part of a PhD program) in another country and continue collaboration in the same area upon return to the home institution.

Session 7: National and International Collaborations with NGOs, Academia, Pharma & Biotech was chaired by **Pawel Kalinski, MD, PhD** and **Leigh Palladino, JD** from Roswell Park. Regulatory, fiscal & logistic arrangements needed to perform collaborative clinical trials with domestic and foreign academic & biotech partners were discussed by **Pawel Kalinski, MD, PhD** & **Donald Handley, MS, MBA** from Roswell. The presenters highlighted recent success of Roswell in replacing Intron-A, discontinued by Merck in 2019, with Argentinian produced generic version of IFNa2b,

Bioferon, which allowed for continuation of over 10 year-long NCI-funded program of Dr. Kalinski and colleagues performed at the University of Pittsburgh and Roswell Park, in collaboration with Merck and Florida-based AIM Immunotech, the supplier of rintatolimod (Ampligen, a TLR3 ligand). The positive results of the resulting phase I clinical trial combining systemic (i.v.) rintatolimod with IFN α 2b in patients with advanced breast cancer, manifested by the safety and average 10-fold in intratumoral CTL markers (Gandhi et al., 2023) can now be extended to the tests of the clinical efficacy of this combination when combined with PD1 blockade, thanks to the recent FDA approval of the use of Bioferon as a part of the experimental chemokine-modulating (CKM) regimen. Dr. Kalinski highlighted the collaborative opportunities for testing CKM in combinations with chemo-, targeted- and radiotherapy in additional cancer types. **Christopher McAleer, PhD** (AIM Immunotech, Ocala, FL) presented the overview of Ampligen's clinical progress in oncology and collaborative opportunities in the areas of the company in pancreatic, breast, ovarian and other cancers. **Rafal Obuchowicz, MD** (Raygenic, Warsaw, Poland) presented the company's unique platforms of image analysis and interests in collaboration with the US partners.

The presentation of **Katie Noyes, PhD, MPH** (State University of New York at Buffalo) addressed the burning topic of overcoming the urban-rural disparities in cancer care delivery. **Michał Mikula, MD, PhD** (NIO, Warsaw) provided an overview of the preclinical resources and unique models available at NIO for collaborative projects with academic, biotech and pharma partners. **Malgorzata Chalupowski, MD, PhD, JD**, and **Piotr Pierog, PhD** from the Copernicus Institute of New England (Cambridge, PA) presented their efforts at bringing together representatives of Biotech and STEM professionals from the Polish-American community. The session was concluded by **Daniel Powell, Jr, PhD** (University of Pennsylvania, Philadelphia, Pennsylvania), the President of the Translational Research Cancer Centers Consortium (<https://www.trccc.org/>) involving 14 cancer centers from North-eastern United States and Canada. Dr. Powell highlighted the history and mission of TRCCC as an organization successfully promoting multi-institutional collaborations between academic and biotech partners, aiming to accelerate the development of new cancer therapies. The next meeting of TRCCC will take place in Seven Springs, Pennsylvania February 21 - 23, 2024 (<https://www.trccc.org/annual-meeting/>), providing ample opportunities for the discussion of partnering options with the Consortium participants.

Session 8: Collaborative Efforts & Opportunities: Experience of Ukraine was chaired by **Mikhail**

Berezin, PhD (Washington University, St Louis, MO) and **Sergii Dubrov, MD, PhD** (First Deputy Minister of Health of Ukraine Kharkiv, Ukraine) and involved **Tetiana Orabina; MBA** (Ministry of Health, Kharkiv, Ukraine), and **Anna Titkova, MBA, MD, PhD** (Kharkiv National Medical University and Pratia Ukraine, Kharkiv, Ukraine).

Dr. Dubrov and colleagues discussed the extraordinary challenges faced by the current healthcare system of Ukraine amidst the war. Speakers presented the overview of the current situation and plans to restore and enhance Ukraine's healthcare infrastructure. The presentations provided prospective directions for the healthcare system reform as well as the past and the future of clinical trials. The presented approach aims to build a resilient and forward-looking healthcare system through collaborations with the neighboring countries and the US. The Ministry of Health of Ukraine has formulated a recovery plan tailored to the context of war and reconstruction. The plan emphasizes the ongoing transformation and improvement of the healthcare system and the continuation of health reforms. The primary objective is to deliver quality services to the public through financially sustainable mechanisms in the medium run. Both the Ministry of Health and local executive authorities are strategically planning to streamline the extensive and fragmented hospital network.

Ukraine has a strong record of clinical trials. Before the war, 794 clinical trials were approved, 2500 research centers were operating and more than 1000 researchers with ICH-GCP standards compliance were working in the country. 24th of February 2022 transiently stopped all clinical trial activities in the country, but that activity was restarted in the following months. The average clinical trials approval time decreased from 47 to 30 days. In 2023, 13 new clinical trials started, 20 studies resumed patient recruitment, 32 patients returned to clinical research sites.

Session 9: Reducing Treatment Burden and Improving Patient Experience was chaired by **Kara Eaton, MA, CPXP**, and **Kathleen Kokolus, PhD**, from Roswell Park. The session represented one of the key areas of focus of the MSCS-CRC Initiative, the quality of life (QOL) of cancer patients and their families, the area with significant differences in emphasis on between the United States and Europe.

Andras Perl, MD, PhD (Upstate Medical University, Syracuse, New York, USA) discussed the growing problem of Immune checkpoint inhibitor (ICI)-induced autoimmune phenomena, reported in 4.2-87.5% of ICI-treated cancer patients with preexisting genetic susceptibility. He discussed the pathogenesis and emerging treatment developed in his and other cen-



Fig. 2. MSC-2023 included unstructured time over meals and session breaks to provide opportunities for networking and small group discussions, similar to the past editions of the MSCS-CRC Symposia, which propelled the collaborative projects presented in Session 1.

ters. **Elizabeth Griffiths, MD** (Roswell Park, Buffalo, NY, USA) presented her experience with the clinical management of myelodysplastic syndrome (MDS), a clonal malignancy of aging patients associated with bone marrow failure, transfusion dependence, decreased life expectancy and risk for transformation to acute myeloid leukemia. The advanced age at presentation (typically in the mid-70s) and frequent existence of comorbidities, limit the application of the only proven curative intervention: allogeneic stem cell transplant. Additional therapies, including transfusion and chemotherapy are largely palliative and current response assessments suggest limited impact on survival for many patients. Since QOL is significantly lower for MDS patients compared to age matched controls and therapy is largely palliative, the ability of treatments to improve the symptoms is of a particular importance. Prior research suggests that QOL is prognostic, even corrected for performance status and conventional clinical staging, and offers an important novel endpoint for clinical trials. Routine application of the now internationally validated MDS-specific QALMs instrument (<https://qualms.dana-farber.org/>) for assessing QOL in MDS will improve our ability to quantify the QOL impact for established and novel

therapy in MDS and thereby maximize outcome for this underserved population.

Anurag Singh, MD (Roswell Park, Buffalo, NY, USA) discussed his highly collaborative work on the negative impact of beta-2 adrenergic stress (Chen et al., 2020a) on the effectiveness of radiation therapy and the patients' QoL. In addition, he presented data on how the baseline quality of life can impact outcomes in H&N (Farrugia et al., 2021a) and lung cancer (Farrugia et al., 2021b). In addition, the impact of financial stress on outcomes in H&N (Ma et al., 2021) and breast cancer (Ma et al., 2023) were discussed. Finally, research on smoking cessation, even if initiated directly prior to treatment, can modify stress in a simple, yet impactful way, influencing survival in H&N (Ma et al., 2022) and lung cancer (Dobson Amato et al., 2015). **Shipra Gandhi, MD** (Roswell Park, Buffalo, NY, USA) discussed the feasibility of replacing the currently used neoadjuvant chemotherapy/ICI combination in patients with high-risk triple-negative breast cancer (TNBC) with a less toxic combination of ICI with CKM regimen, combining IFN α 2b with rintatolimod. Her preliminary data from the clinical trial NCT04081389 investigating increasing doses of IFN along with fixed dose rintatolimod and celecoxib (CKM) in addition

to standard neoadjuvant chemotherapy (paclitaxel, doxorubicin, and cyclophosphamide) in stage I-II triple negative breast cancer showed residual cancer burden (RCB-0 and 1) rate of 66% (6/9 patients), comparable to the one expected on chemotherapy/ICI combination. No dose limiting toxicities were observed. Adverse events attributed to CKM were mostly limited to grade 1-2 toxicities. Future trials will test CKM and chemotherapy along with neoadjuvant pembrolizumab in triple negative breast cancer.

Kara Eaton, MA, CPXP (Roswell Park, Buffalo, NY, USA) concluded the session with the presentation of her data that making patients the center of care in hospitals is not only the right thing to do, but it also that leads to improved health outcomes, increased patient satisfaction, and more efficient healthcare delivery. This holistic approach to healthcare sees patients as more than their diagnosis, but rather unique individuals with physical, emotional, and social complexities. Recognizing the entire patient experience is fundamental in providing compassionate, safe and effective healthcare. The novel approaches to achieve this goal successfully introduced at Roswell can be rapidly implemented in other centers, representing a low-cost way to enhance both the satisfaction of pa-

tients and their families and to improve the outcomes of cancer patients' treatment.

ACTION ITEMS AND PLANS FOR MSC-2024

The discussions over MSC-2023 led the identification of the key areas of priorities (Figure 1) for MSCS-CRC Initiative to be pursued over the next year and the following year. The immediate efforts are a) the development of the data base helping our existing and new participants to identify collaboration partners for the existing and upcoming projects and the available sources of funding. The second area of our immediate efforts is the pursuit of stable funding to support the upcoming conferences, to allow increased involvement of junior faculty and trainees (travel grants for selected presenters) and invite high visibility speakers in the selected areas of priority. The next Symposium MSC-2024 will be held in Buffalo, September 22-25, 2024, with the 2025 event to be held at the NIO in Warsaw Poland, the 2026 meeting at Moffitt Cancer Center, Tampa, Florida. Prospective meetings hosted by additional institutions, are discussed with participants representing Washington University, Purdue University, City of Hope, as well as colleagues from Croatia and Ukraine.

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ORIGINAL ARTICLE

VULVOVAGINAL CANDIDIASIS AFTER GYNECOLOGICAL SURGERIES AND ADVERSE PREGNANCY OUTCOME IN UKRAINE: A MULTICENTRE STUDY

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ABSTRACT

The aim: To investigate the epidemiology and microbiology of vulvovaginal candidiasis (VVC) after gynecological surgeries, and adverse pregnancy outcomes in Ukraine.

Materials and methods: Multicenter prospective cohort study was conducted from January 2020 to December 2022 and recruited pregnant and non-pregnant women aged 15–65 years who had sought medical help for vaginal dysbiosis the seven medical clinic from five regions of Ukraine.

Results: Between 2020 and 2022, 2,341 women were followed in gynecological practices, and 1,056 (41.5%) women were diagnosed with VVC during the same period. Of the total VVC cases, 31.9% were in non-pregnant and 68.1% in pregnant women. The use of antibiotics (OR=3.48), use hormonal contraceptives (OR=2.75) and pregnancy (OR=1.13) were associated with an increase in the risk of VVC diagnosis. Diabetes mellitus (OR=0.44) were additional risk factors. The most common pathogen of VVC was *C. albicans*, *Nakaseomyces glabratus* (*C. glabrata*), followed by *Pichia kudriavzevii* (*C. krusei*), *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. guilliermondii*, *C. lusitanae*, and *C. rugosa*. We found no significant difference in adverse pregnancy outcomes between *Candida*-positive and *Candida*-negative women.

Conclusions: Vulvovaginal candidiasis after gynecological surgeries in Ukraine is a common medical problem in women that is associated with significant morbidity, and hence frequent medical visits. High prevalence rate of vulvovaginal candidiasis in the present study warrants, the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to non-albicans *Candida* species in Ukraine.

KEY WORDS: Prevalence, vulvovaginal candidiasis, responsible pathogens, risk factors, adverse pregnancy outcomes, Ukraine

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INTRODUCTION

Vulvovaginal Candidiasis (VVC) affects millions of women every year and has been considered an important public health problem. Although not associated with mortality, the morbidity associated with VVC makes it a major cause of mental distress, causing pain, great discomfort, altered self-esteem, anxiety, impairing work performance and interfering with sexual and affective relations. Despite therapeutic advances, VVC remains a common problem worldwide, affecting all strata of society [1].

According to literature, 75 % of all women will experience at least one episode of VVC in their lives. Between

40 % and 50 % of initially infected women will experience a second episode. In Europe VVC is one of the most common causes of vaginitis, and in the United States, it is the second most frequent cause of infection after bacterial vaginosis [2,3]. Literature evidence regarding VVC reports highlighted that this disease it is the second most common infection of the vulvovaginal area of symptomatic women accounting for about 17% to 42% [4-6]. Vulvovaginal yeast infections in pregnancy are common and can cause extensive inflammation, which could contribute to adverse pregnancy outcomes [7].

Candidiasis is an infection caused by a yeast (a type of fungus) called *Candida*. *Candida* normally lives on skin

and inside the body such as in the mouth, throat, gut, and vagina, without causing any problems. *Candida* can cause an infection if conditions change inside the vagina to encourage its growth. The common term for candidiasis in the vagina is a vaginal yeast infection. Other names for this infection are vaginal candidiasis, vulvovaginal candidiasis, or candidal vaginitis.

Healthcare providers usually diagnose vaginal candidiasis by taking a small sample of vaginal discharge. They examine the sample under a microscope in the medical office or send it to a laboratory for a fungal culture. However, a positive fungal culture does not always mean that *Candida* is causing symptoms. Some women can have *Candida* in the vagina without having any symptoms. Vaginal candidiasis is often mild. About 20% of women normally have *Candida* in the vagina without having any symptoms [2].

Analysis of previous studies showed that understanding of anti-*candida* host defence mechanisms in the vagina has developed slowly and, despite a growing list of recognised risk factors, a fundamental grasp of pathogenic mechanisms continues to elude us. The absence of rapid, simple, and inexpensive diagnostic tests continues to result in both overdiagnosis and underdiagnosis of VVC. Currently, VVC in Ukraine is routinely diagnosed by sign and symptom and is not confirmed with laboratory investigation when necessary. As a result, the spectrum of yeasts implicated in causing VVC, their drug susceptibility profile is not known in the country. In Ukraine, similar studies focused on VVC have not been carried out.

THE AIM

The aim of this study was to investigate the epidemiology and microbiology of VVC after gynecological surgeries, and adverse pregnancy outcomes in Ukraine.

MATERIALS AND METHODS

DESIGN, SETTINGS AND STUDY PARTICIPANTS

Multicenter prospective cohort study was conducted from January 2020 to December 2022 and recruited pregnant and non-pregnant women aged 15–65 years who had sought medical help for vaginal dysbiosis the seven medical clinic from five regions of Ukraine. The study population included women who had history of surgery for gynecological disease. All pregnant women in any trimester who visited the antenatal clinics were interviewed one-on-one and those that gave consent to be part of the study were enrolled. Ex-

clusion criteria included, Chlamydial infections, Syphilis or other sexually transmitted bacterial infections, had bacterial and aerobic vaginosis. Pregnant women who had any pregnancy related complications (diabetes, bleeding per vagina, hypertension) were excluded in the study. Pregnant women were enrolled once to avoid repetition.

DEFINITION OF VULVOVAGINAL CANDIDIASIS

Symptomatic vulvovaginal candidiasis was defined as the presence of clinical presentations and the presence of yeast-liked cells in direct Gram smear with positive culture.

DEFINITION ADVERSE PREGNANCY OUTCOME

Relevant adverse pregnancy outcomes were based on the core outcome set for preterm birth (PTB) research and cross-checked with other core outcome sets in women's and newborn's health. The main outcomes were as follows: PTB, defined as birth at <37 weeks' gestation; spontaneous PTB, defined as birth at <37 weeks' gestation after spontaneous onset of labor or after Preterm prelabor rupture of membranes (pPROM); small for gestational age, defined as birthweight below the 10th percentile for gestational age; pPROM, defined as preterm prelabor rupture of membranes at <37 weeks' gestation; and neonatal death, defined as death during the first 28 days of life. Additional outcomes were as follows: late pregnancy loss, defined as birth between 20- and 24-weeks' gestation; early pregnancy loss, defined as spontaneous demise of pregnancy at <20 weeks' gestation; prelabor rupture of membranes (PROM), defined as rupture of membranes before the onset of labor; birthweight; maternal mortality; maternal morbidity; and neonatal morbidity. Because of the lack of consensus in defining stillbirth, all definitions used for stillbirth were included.

DATA COLLECTION

Information regarding participants' socio-demographics (age, educational level, marital status, trimester in pregnancy) and microbiological data, parity, history of surgical procedure, preoperative and postoperative antibiotic use, complications, and clinical presentations were extracted from participants' ambulatory medical records and relevant hospital records medical records. Information that was not readily available in the medical records were collected using structured questionnaire

Table I. Characteristics study participants (n=2341) with a diagnosis of vulvovaginal candidiasis (VVC) and without VVC in Ukraine, 2020-2022

Variable	Number of women (n=2341)	Vulvovaginal candidiasis		P value
		Yes (n=1056)	No (n=1285)	
Age (years), n (%)				0,062
<18	236	155 (14.7)	81 (6.3)	
18-25	547	411 (38.9)	136 (10.6)	
26-30	712	211 (20.0)	501 (39.0)	
31-35	378	55 (5.2)	323 (25.1)	
36-40	214	86 (8.1)	128 (10.0)	
41-50	189	94 (8.9)	95 (7.4)	
51-60	47	35 (3.3)	12 (0.9)	
>60	18	9 (0.9)	9 (0.7)	
Marital status, n (%)				0.052
Married	786	153 (14.5)	633 (49.3)	
Unmarried	1023	601 (56.9)	422 (32.8)	
Divorced	532	302 (28.6)	230 (17.9)	
Education level, n (%)				0.083
Secondary school	1,243	331 (31.3)	912 (71.0)	
College	481	284 (26.9)	197 (15.3)	
High	617	441 (41.8)	176 (13.7)	
Smoking habits, n (%)	2,296	1,029 (97.4)	1,267 (98.6)	0.362
Alcohol consumption, n (%)	1,535	659 (62.4)	876 (68.2)	0.128
Place of residence, n (%)				0.311
Urban	1,467	659 (62.4)	808 (62.9)	
Rural	874	397 (37.6)	477 (37.1)	
Pregnant status, n (%)				<0.001
Non-pregnant	1,507	337 (31.9)	1,170 (91.1)	
Pregnant	834	719 (68.1)	115 (8.9)	
Multifetal pregnancy, n (%)	436	37 (3.5)	399 (31.0)	0.39
Use hormonal contraceptives, n (%)	756	563 (74.5)	193 (25.5)	<0.001
Weakened immune system (steroid use), n (%)	258	189 (73.3)	69 (26.7)	<0.001
Antibiotic use, n (%)				<0.001
Preoperative antibiotics not given	327	25 (7.6)	302 (92.4)	
Preoperative antibiotics given	921	199 (21.6)	722 (78.4)	
Long-term use of antibiotics	518	364 (70.3)	154 (29.7)	
History of diabetes mellitus, n (%)	168	117 (69.6)	51 (30.4)	0,002
History of adverse pregnancy outcomes, n (%)				0.125
Preterm birth (PTB)	131	19 (14.5)	112 (85.5)	
Spontaneous PTB	127	49 (38.6)	78 (61.4)	
Small for gestational age	211	42 (19.9)	169 (80.1)	
Preterm prelabor rupture of membranes	138	34 (24.6)	104 (75.4)	
Late pregnancy loss	109	18 (16.5)	91 (83.5)	
Early pregnancy loss	98	21 (21.4)	77 (78.6)	
Prelabor rupture of membranes	132	34 (25.8)	98 (74.2)	
Low birth weight	219	26 (11.9)	193 (88.1)	

Table II. Risk factors for VVC in study participants in Ukraine, 2020-2022

Variable	Univariable analysis		Multivariable analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
Age (years)	0.972 (0.944-1.004)	0.062		
Antibiotic use	3.476 (2.226-5.427)	<0.001	2.506 (1.536-4.083)	<0.001
Use hormonal contraceptives	2.754 (1.743-4.355)	<0.001	2,616 (1,638-4,174)	<0.001
Pregnancy	1.132 (1.092-1.172)	<0.001	1.142 (1.098-1.185)	<0.001
Diabetes mellitus	0.438 (0.257-0.746)	0.002	0.428 (0.248-0.738)	0.002

OR, Odds Ratio; CI, Confidence Interval.

Table III. Distribution of *Candida* isolates from 2341 patients with vulvovaginal candidiasis (VVC) in Ukraine, 2020-2022

Species of <i>Candida</i>	2020 (n=754)		2021 (n=770)		2022 (n=814)		Total (n=2341)		Trend (↑↓)
	n	%	n	%	n	%	n	%	
<i>C. albicans</i>	591	78.4	609	79.1	627	77.0	1827	78.0	↓
<i>Nakaseomyces glabratus</i> (<i>C. glabrata</i>)	35	4.6	41	5.3	43	5.3	119	5.1	↑
<i>Pichia kudriavzevii</i> (<i>C. krusei</i>)	33	4.4	27	3.5	43	5.3	103	4.4	↑
<i>C. parapsilosis</i>	29	3.8	21	2.7	22	2.7	72	3.1	↓
<i>C. tropicalis</i>	17	2.3	23	3.0	29	3.6	69	2.9	↑
<i>C. kefyr</i>	20	2.7	20	2.6	20	2.5	63	2.7	↓
<i>C. guillieromondii</i>	13	1.7	10	1.3	10	1.2	33	1.4	↓
<i>C. lusitaniae</i>	8	1.1	12	1.6	6	0.7	26	1.1	↓
<i>C. rugosa</i>	6	0.8	4	0.5	7	0.9	17	0.7	↑
<i>C. famata</i>	2	0.3	3	0.4	7	0.9	12	0.5	↑

administered to participants of the study. All women were assigned study identification numbers (IDs) which were used throughout the study. The IDs were generated by using the initials of the hospital where participants were enrolled and a chronological number according to how the patients were recruited.

ETHICS

The study received ethics approval from the Shupyk National Healthcare University of Ukraine, Kyiv, Ukraine. The present study was performed in line with the principles of the Declaration of Helsinki.

MICROBIOLOGICAL ANALYSIS

After detailed explanation of the sampling procedure to the participants, two high vaginal swabs were obtained by a Gynecologist/trained midwife from each participant using sterile swab sticks. Specimens were taken from the posterior fornix of the vagina with sterile swabs and transported to the microbiology laboratory for further processing. Identification to the species level was performed by MALDI-TOF MS (Bruker Daltonics) and bacterial strains by Vitek 2 automated system (BioMérieux). For quality control, *C. albicans*

(ATCC 10231) was used as reference strain and tested simultaneously with the clinical isolates.

STATISTICAL ANALYSIS

All the analysis were carried out using SAS 9.4 (SAS Institute, Cary, NC, USA). The prevalence of VVC, defined as the proportion of women diagnosed with VVC (denominator: all women who visited the gynecological practices), was analyzed. Categorical data variables were statistically described in the form of frequencies and percentages while continuous data variables were summarized as mean (Standard Deviation). The association between categorical variables were done using chi-square test. The association between VVC diagnosis and the predefined variables was studied using a bivariate analysis and multivariate logistic regression model. P-value less than 0.05 was considered significant.

RESULTS

PREVALENCE OF VVC

During the study period (2020-2022), 1056 of 2341 women's undergoing gynecologic surgery were found to have Vulvovaginal Candidiasis (VVC). The prevalence

of VVC among study participants was 45.1% (95% CI: 44.1–46.1, $p < 0.001$). Of the total VVC cases, 31.9% were in non-pregnant and 68.1% in pregnant women. The highest prevalence rates of VVC were found in the age groups of 18–25 years (38.9%), 26–30 years (30.0%), and <18 years (14.7%), respectively. Demographic and clinical characteristics of study participants with a diagnosis of vulvovaginal candidiasis (VVC) and without VVC are shown in Table I.

RISK FACTORS FOR VVC

The results of the multivariate logistic regression model are displayed in Table II. The use of antibiotics (OR=3.48), use hormonal contraceptives (OR=2.75) and pregnancy (OR=1.13) were associated with an increase in the risk of VVC diagnosis. Diabetes mellitus (OR=0.44) were additional risk factors.

RESPONSIBLE PATHOGENS

In this study after microbiological analyzes 2341 isolates were recovered from the clinical samples. Assessing the prevalence of the identified subtypes, the predominance of *C. albicans* species was found (78.0%), compared to non-albicans *Candida* species (22.0%). The most common non-albicans species were *Nakaseomyces glabratus* (*C. glabrata*) (5.1%) followed by *Pichia kudriavzevii* (*C. krusei*) (4.4%), *C. parapsilosis* (3.1%), *C. tropicalis* (2.9%), *C. kefyr* (2.7%), *C. guilliermondii* (1.4%), *C. lusitanae* (1.1%), *C. rugosa* (0.7%) and other species ($n=12$; 0.5%). Of the total number of 2341 *Candida* isolates, 1325 (56.6%) showed bacterial associations. The most common microbial association was *C. albicans* and *S.aureus* - 127 combinations (26.6%), followed by *C. albicans* and *E.coli* - 99 combinations (20.8%). Bacterial associations with non-albicans *Candida* species or recorded less frequently. Distribution of *Candida* isolates from patients with VVC during study period (2020–2022) in Ukraine showed in table III.

ADVERSE PREGNANCY OUTCOMES

In this study we found no significant difference in preterm birth (PTB) rate, spontaneous PTB, small for gestational age, preterm prelabor rupture of membranes (pPROM), late pregnancy loss, early pregnancy loss, prelabor rupture of membranes (PROM), and low birth weight between *Candida*-positive and *Candida*-negative women. Characteristics of study participants with history adverse pregnancy outcomes are shown in Table I. Logistic analyses for history adverse pregnancy outcomes among 2,341 women did not reveal any statistically significant associations either.

DISCUSSION

The results of present study provide data as first research in Ukraine that focuses on epidemiology, microbiology, and adverse pregnancy outcomes in Ukraine. Information regarding the prevalence of VVC in Ukraine is not well known.

VVC is not a reportable disease, and therefore, the information on its incidence is incomplete and based on epidemiology studies that are often hampered by inaccuracies of diagnosis and/or the use of non-representative populations. The prevalence of vulvovaginal candidiasis varies from one study to another. Multiple previous studies showed the prevalence rate of VVC among reproductive age women varies between countries and different regions, ranging from 12 to 72% [1, 4–6, 8, 9]. The reasons for such varying prevalence of VVC might be explained the investigation of different geographical locations, profile of the population being studied and period of time in these studies. In this study, the incidence of VVC in Ukraine was found to be 45.1%.

The development of VVC is usually attributed to the disturbance of the balance between *Candida* vaginal colonization and host environment by physiological or nonphysiological changes. The risk factors are believed to be associated with increased rate of VVC including host-related factors such as hyper-estrogenic state (pregnancy, hormone replacement therapy), poorly controlled diabetes, immunodeficiency states, use of antibiotic, treatment with glucocorticoids and genetic predispositions and behavioral factors such as birth control pills, intrauterine device, spermicides and condoms and hygiene habits, tight-fit clothing and sexual behaviour [1]. Other studies have shown that the major risk factors for the development of VVC are lifestyle-related (e.g., frequency of sexual intercourse, contraception, or vaginal douching) [10, 11]. Despite a growing list of recognized risk factors, much remains to be elucidated. In our study the use of antibiotics, use hormonal contraceptives and pregnancy were associated with an increase in the risk of VVC diagnosis, and diabetes mellitus were additional risk factors VVC diagnosis was positively associated with antibiotic prescriptions, prescription of contraceptives, and pregnancy (Table III).

The present study of the total VVC cases, 31.9% were in non-pregnant and 68.1% in pregnant women. Pregnancy and hormone replacement therapy pregnancy has been considered an important risk factor for the development of VVC because several studies report high incidence of the disease in pregnant women. According to literature, VVC caused by *Candida* species, are more common in pregnant women than non-pregnant women [12–14], potentially because

of hormonal and immunological changes that occur during pregnancy [15]. The epidemiologic studies have been consensual in reporting higher prevalence of the disease in pregnant women than in non-pregnant patients, although the incidence varies depending on the locations. The high incidence of VVC in pregnancy has been attributed to the increase of sex hormones secretion in pregnancy. Vulvovaginal candidiasis higher in the last trimester, when levels of hormones are more elevated, even though symptomatic recurrences are common throughout pregnancy [16, 17]. Furthermore, in nonpregnant women the infection is more incident during the luteal phase of the menstrual cycle, which is the phase with the highest hormone.

Candida albicans is the leading cause of vulvovaginal yeast infections; however, other species are becoming relevant in this niche. The spatial distribution of these fungi in the female genital tract remains poorly understood [18]. *Candida* spp. constitute one of the most important genus of opportunistic pathogenic fungi in humans [19, 20], comprising the great majority of isolates obtained from fungal invasive and mucosal infections [20, 21]. Globally, the five species that belong to the genus *Candida*, presently or formerly, that are more commonly associated with candidosis in humans are *C. albicans*, *Nakaseomyces glabratus* (formerly known as *C. glabrata*), *C. tropicalis*, *C. parapsilosis*, and *Pichia kudriavzevii* (formerly known as *C. krusei*) [22, 23]. In our study the most common species were *C. albicans*, *Nakaseomyces glabratus* (*C. glabrata*), followed by *Pichia kudriavzevii* (*C. krusei*), *C. parapsilosis*, *C. tropicalis*, *C. kefyr*, *C. guilliermondii*, *C. lusitaniae*, and *C. rugosa*.

Present study demonstrates the necessity of identification the *Candida* species responsible for infections in all patients presenting with VVC especially those with recurrent infections. Therefore, identification will influence selection of antifungals and duration of therapy. Simultaneously, compared to previous years, a trend has been observed and that is an increase in the rate of isolation of non-albicans *Candida* species. This phenomenon could be due to the improvement of laboratory diagnostic practices regarding the identification of fungi or a real higher prevalence of these species. The variety of non-albicans *Candida* species involved in human pathology, their rising contribution to fungal infections and the antifungal susceptibility profiles makes their identification at the species level essential for epidemiological investigations, optimizing therapy and patient management.

Recognition that ascending infection leads to adverse pregnancy outcomes has led to a number of studies that have evaluated the treatment of vaginal infections in pregnancy to reduce preterm birth rates. However, the role of candidiasis is relatively unexplored. Some

studies suggest an association between vaginal *Candida* colonization and adverse pregnancy outcomes, but the evidence is inconsistent [2, 23-25]. In this study we found no significant difference in adverse pregnancy outcomes between *Candida*-positive and *Candida*-negative women.

STRENGTH AND LIMITATION

To our knowledge, no study addressing of the VVC after gynecological surgeries and risk factors for VVC, and associated adverse pregnancy outcomes. The results of our study provide valuable data as first research in Ukraine and potential for comparison with data from other countries. We recognize that there are some limitations to our study. We were not able to perform analysis on several outcomes, such as late pregnancy loss, spontaneous demise of pregnancy at <20 weeks' gestation, maternal mortality, maternal morbidity, and neonatal morbidity. It is possible that VVC is associated with PTB or another adverse pregnancy outcome with bigger sample size.

CONCLUSIONS

The present study showed that VVC in Ukraine is a common medical problem in women that is associated with substantial discomfort, significant morbidity, and hence frequent medical visits. High prevalence rate of VVC in the present study warrants, the importance of conducting continuous epidemiological surveys to measure changes in species distribution from *C. albicans* to non-albicans *Candida* species in Ukraine. We did not find strong statistical evidence of an increased risk for preterm birth or eight other adverse perinatal outcomes, in pregnant women with either symptomatic or asymptomatic vulvovaginal VVC. The available evidence is insufficient to make recommendations about testing and treatment of vulvovaginal yeast infection in pregnancy. Future studies should assess vulvovaginal symptoms, yeast organism loads, concomitant vaginal or cervical infections, and microbiota using state-of-the-art diagnostics. Use of molecular diagnostic methods would allow accurate detection and quantification of organism load to determine whether the presence of symptoms is associated with higher organism load, and whether higher organism loads are associated with a higher risk for adverse pregnancy outcomes. VVC cannot be seen in isolation and comprehensive evaluation of the role of concomitant vaginal or cervical infections, or certain microbiota should also be investigated in holistic studies. Additional studies on a larger sample and evaluating results of antifungal susceptibility testing are of great importance for optimizing therapy and patient management to fighting VVC with targeted therapy.

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ORIGINAL ARTICLE

RELATIONSHIP OF DERMATOGLYPHICS WITH SPEED OF REACTION AND TYPE OF TEMPERAMENT OF ATHLETICS

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ABSTRACT

The aim: To establish and practically substantiate the relationship between dermatoglyphic markers and the speed of reaction and type of temperament of track and field athletes.

Materials and methods: Analysis of scientific and methodological literature, survey, dermatoglyphics, methods of mathematical and statistical processing of the received data.

Results: Track and field athletes among the population of the Sumy region of Ukraine have a tendency to decrease the number of whorls ($p < 0.05$). Athletes of the sanguine type of temperament are most common (67,6%), and in the control group (among students of a medical university) the phlegmatic type of temperament is most common (68,4%). Also, among the subjects of the main group there is no such temperament as melancholic. We also determined the ATD angle for the palm. It was 37 ± 4.88 in the main group, and 47 ± 3.11 in the control group. This indicates a hereditary predisposition of this trait. The delta index in the control group has lower values ($DI=9.5$) than in the main group ($DI=13.3$).

Conclusions: We established and practically substantiated the relationship between dermatoglyphic markers and reaction speed and temperament type of track and field athletes. Determined the relationship between the anatomical features of the fingers and the speed of mastering movements. For track and field athletes of the population of Ukraine, there were characteristic features of the dermatoglyphic structure: higher values of the deltoid index and genetic markers of the distance between the triradii a and d of the fingers.

KEY WORDS: genetic marker, dermatoglyphics, athlete, temperament, finger patterns

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INTRODUCTION

Dermatoglyphics is a branch of human morphology that studies the skin relief of palmar and plantar surfaces, where the skin is covered with numerous combs (papillary lines) that form certain patterns [1]. Combs are linear thickenings in depth and on the surface of the epidermis. Papillary lines and patterns do not change with age, are not influenced by environmental conditions and are characterized by great individual variability. Many of their features are passed on to descendants [2].

Dermatoglyphics are patterns on the pads of the fingers and toes. They, like the patterns on the palms and feet, are one of the most important individual characteristics of a person. Dermatoglyphic indicators are one of the most accessible genetic markers for study and use. Finger patterns are an invariable genetically determined hereditary feature during life, formed in 3-5 months of pregnancy, have structural diversity and high individual group variability [3, 4].

The secret of dermatoglyphics is extremely deep. The study of finger patterns will make it possible to determine whether there is a connection between heredity and types of temperament. Today it is known that every person has hidden resources and potential for the development of individual abilities and physical qualities [5-9]. An urgent issue today is the determination of the most optimal ways of developing individual abilities and their forecasting.

It is also worth noting that benign forms with finger patterns are extremely important and valuable material, because they can be used for other research in the future. Therefore, the study of finger patterns in individuals with different types of temperament will make it possible to reach a certain consensus on the issue of the influence of heredity on the manifestation of physical qualities of track and field athletes, which causes a large number of discussions in scientific circles.

In the world, there is a growing need for quick and effective prediction of future athletes, which can be provided by studying genetic markers (finger patterns,

ATD angle for the palm, delta index (DI), the distances between the triradii a and d, c). Sports performance is a complex phenomenon that includes will, discipline, training, psychological state, ability to work in a team and many other aspects. Literature suggests, that the study of genetic markers can provide additional information about physiological aspects that can influence sports potential [5-9], can also reliably predict the future sports potential or achievements of athletes. So, genetics is one of the factors for predicting future sports success by genetic markers.

In the future, genetic research can provide valuable data to improve the effectiveness of training, personalization of the approach to sports and health. But in order to fully predict sports potential, it is important to combine genetic data with other important factors, such as physical training, psychological state, support and others. Indeed, genetic factors can influence some aspects related to physical abilities, such as muscle mass, reaction speed, visibility, height, etc. However, the determination of athletic potential using genetic markers can be a challenging task due to the large number of genetic interactions and the influence of external factors that can alter the detection of these markers. This question needs further research.

During our study, we established and practically substantiated the relationship between dermatoglyphic markers and speed of reaction and type of temperament of track and field athletes. According we determine the most common dermatoglyphic markers of the control and main group of subjects; to find out the relationship between dermatoglyphic markers and the type of temperament of track and field athletes; to determine the values of the ATD angle and the delta index for the palm of athletes-athletes among the population of Ukraine; to determine the relationship between dermatoglyphic markers and the prediction of performance in athletics.

The object of the study is the dermatoglyphic markers of students and track and field athletes with different types of temperament. The subject of the research is the study of dermatoglyphic markers in people with different types of temperament.

THE AIM

The purpose of our study was to establish and practically substantiate the relationship between dermatoglyphic markers and the speed of reaction and type of temperament of track and field athletes.

MATERIALS AND METHODS

The research sample comprised of 76 medical students aged 17–20 (control group) and 68 track and field athletes aged 18-25 (main group). Both the main and

control groups had only men in their composition. The control group included ordinary first-year students of the medical institute without concomitant diseases. The main group included track and field athletes with the rank of candidate for master of sports (50%) and master of sports of Ukraine (50%).

Before the start of the research, students and track and field athletes were familiarized with the goals and hypothesis of the research, and consent to the research was obtained. The hypothesis of our research is the detection of finger patterns in athletes with different types of temperament, which will make it possible to reach a certain consensus on the issue of the influence of heredity on the manifestation of physical qualities of track and field athletes, which provides a large number of discussions in scientific circles. Temperament determines the strength and speed of reactions to life events, its degree of manifestation in sports activities. In this way, by establishing a connection between temperament and such a genetic marker as dermatoglyphics, we will predict the effectiveness of forecasting in the selection of future track and field athletes. This is realized in increasing sports results, reducing financial costs for training athletes.

This study consisted of a one-time data collection (fingerprinting) and a temperament survey of respondents. The study was conducted at two universities of Ukraine: Sumy State University and Makarenko Sumy State Pedagogical University. The questionnaires were distributed in paper form and respondents were instructed on how to complete it and informed of survey questions related questions about the definition of temperament. The work was performed in accordance with the principles Declaration of Helsinki of the World Health Organization association «Ethical principles of medical investigations involving a person as an object research» and approved by the Bioethics Commission Sumy State Medical Institute. All participants provided informed consent to participate in this study and all of the procedures were approved by the ethics committee of the Sumy State University.

To diagnose a person's temperamental features, a questionnaire developed by V. M. Rusalov, based on his own concept of temperament, was used [10]. Students are asked to answer 105 questions, which are aimed at finding out the usual way of behavior. You need to answer quickly and accurately. Keys are used to calculate points on one or another scale. The conclusion about the dominant type of a person's temperament is made on the basis of a comparison of indicators obtained from different temperament properties with the typical combinations of these properties corresponding to different types of temperament given below. San-

Table I. Number of finger patterns in both groups, %

groups	Loop (%)				Arch (%)		Whorl (%)	
	Ulnar (U)		Radial (R)		Right	Left	Right	Left
	Right	Left	Right	Left				
main	6,5±0,02	19,5±0,07	30±0,23	23±0,3	4±0,06	6±0,04	6±0,05	5±0,06
	26		53		10		11	
control	13±0,25	25±0,13	22±0,03	3±0,22	3±0,06	6±0,03	13±0,15	15±0,13
	38		25		9		28	

Table II. Relationship between finger patterns and temperament

Temperament	Arch				Whorl				Loop			
	min	max	μ	σ	min	max	μ	σ	min	max	μ	σ
control group												
Choleric (n=9)	3	10	5,7	2,22	2	5	3,44	1,28	9	13	10,6	1,24
Melancholic (n=3)	7	7	7	0	1	2	1,5	1,18	10	11	10,5	0,22
Sanguine (n=12)	6	10	7,7	1,87	0	2	0,87	0,61	9	13	10,8	1,35
Phlegmatic (n=52)	1	2	1,5	1,18	8	14	11	2,28	9	13	10,6	1,24
main group												
Choleric (n=16)	6	10	7,7	1,87	8	14	11	2,28	9	13	10,6	1,24
Sanguine (n=46)	5	8	6,4	1,6	5	6	5,5	1,61	9	17	12,5	2,29
Phlegmatic (n=6)	1	2	1,5	1,25	4	4	4	1,1	3	10	7,1	1,87

guine is a moderately developed indicator in terms of all temperament properties. Choleric - high indicators of energy, pace and emotionality with average or high indicators of plasticity. Phlegmatic - low indicators in all properties of temperament. Melancholic - low indicators of energy, plasticity, pace with average or high indicators of emotionality.

Technique for the study of sports dermatoglyphics is very basic, consisting of taking fingerprints on pre-established paper formats or direct observation with a magnifying glass. For dermatoglyphic studies, the method of removing impressions of the surfaces of the phalanges of the fingers, using gouache, was used. A sponge and white sheets of A4 format were used to remove prints. Paints or gouache are diluted to the consistency of thick sour cream. The paint is diluted on glass, then rolled onto a sponge, with which the dye is evenly applied to the fingers. Press each finger separately, rolling it on paper from the radial side to the ulnar side. An important practical place here is the clamping force. According to the method, the paint should be applied only to the tops of the crests of the finger patterns, for clarity and recognition of future drawings. A 4 x 7 cm sponge was used to apply paint to the palm.

The next step was to transfer the paint from the fingers to specially prepared questionnaires. This type

of form contains in its structure places for actual fingerprints, as well as individual data of each subject. Moreover, the drawings from the right and left hands are located separately. This makes it possible to more easily navigate the subsequent statistical analysis by comparing the results. We would also like to draw your attention to the fact that when transferring the paint to the form, it is advisable to place a resilient surface under it to obtain clearer results. The obtained data were analyzed statistically.

The following methods were used during the research: analysis of scientific and methodological literature, survey, dermatoglyphics, methods of mathematical and statistical processing of the received data. Statistical data processing was carried out on a personal computer using standard Statgraphics and STATISTICA programs. The Excel package was used for the initial preparation of tables and intermediate calculations. All clinical data were entered in an Excel database for statistical analysis. Results are expressed as median (range), mean ± standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. We compared fingerprints, dermatoglyphic indicators with temperament in the studied and control groups, and determined the hereditary predisposition of the traits. All statistical analyses were two-sided and significance was set at $P < 0.05$.

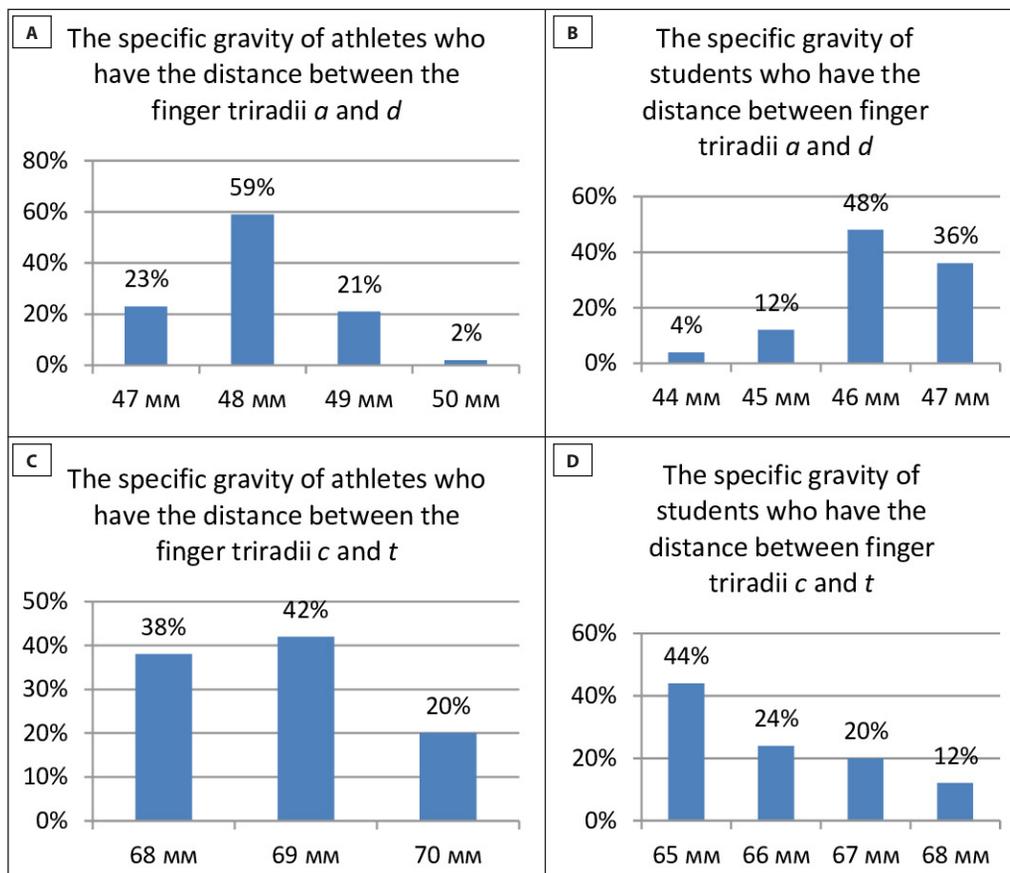


Fig. 1. The specific gravity of persons who have the distance between the triradii of the fingers: A) The specific gravity of athletes who have the distance between the finger triradii a and d; B) The specific gravity of students who have the distance between the finger triradii a and d; C) The specific gravity of athletes who have the distance between the finger triradii c and t; D) The specific gravity of students who have the distance between the finger triradii c and t;

RESULTS

When analyzing the data, after recognizing the types of fingerprints, we found that the most common fingerprint is a loop in both groups. It occurs with a frequency of approximately 79 % in the main group and 63% in the control group; whorl a frequency of 11 % in the main group and 28 % in the control group; arches – 10 % in the main group and 9 % in the control group (Table I). Our research confirms that the radial loop is also related to a high sports performance. We can also say that the physical quality of speedis determined by one delta of loops. This is confirmed by research D. Chapa-Guadiana [11].

According to the results of the study, we determined that the main group of athletes has 17% less whorls than the control group. Thus, we can conclude that the genetic markers of athletes have a higher percentage of such finger patterns as radial loops, fewer whorls and very little archs, unlike the control group. Normally for the Ukrainian population, the number of whorls is 30 - 40%. That is, track and field athletes among the population of the Sumy region of Ukraine have a tendency to decrease the number of whorls ($p < 0.05$).

According to the Eysenck method, the type of temperament was determined in the control and main groups [12]. Sanguine has a strong, lively, balanced type of nervous system (mobile); phlegmatic – strong, calm, balanced type of nervous system (inert); choleric

– mobile, unrestrained, strong type of nervous system (unbalanced); melancholic – an unbalanced, inactive type of nervous system (weak).

We determined that among the subjects of the main group, athletes of the sanguine type of temperament are most common (67,6%), and in the control group (among students of a medical university) the phlegmatic type of temperament is most common (68,4%). Also, among the subjects of the main group there is no such temperament as melancholic, in the control group there are also very few of them – 3,9%.

According to the obtained data, it was determined which finger patterns are characteristic for each type of temperament (Table II). The most extended patterns are loops (L), which occur with almost equal frequency in all temperament types, as well as in the main such and control groups. Such a pattern as a whorls (W) occurs in choleric, in melancholics it occurs very weakly and only on one hand, and in phlegmatics it is absent in the control group. In phlegmatic patients, only such a finger pattern as loops is found, there are very few archs ($p < 0,001$).

No melancholic temperament type was found among those studied in the main group. The remaining types of finger patterns were observed in the ratio that we see in Table II. Loops are also the most common patterns among people of the main group.

We also determined the ATD angle for the palm, which exists between the triradii A, D and T. This angle should not exceed 57°. It was 37 ± 4.88 in the main group, and 47 ± 3.11 in the control group. This indicates a hereditary predisposition of this trait.

We also determined the delta index (DI). The delta index in the control group has lower values (DI=9.5) than in the main group (DI=13.3). Thus, the delta index of track and field athletes among the population of Ukraine is 19% higher than that of students. This also indicates a tendency to increase the delta index in athletes.

The relationship between dermatoglyphic markers and the prediction of performance in athletics was determined. By measuring the distances between the triradii a and d, c and t, we confirmed L.P. Sergienko's [13] conclusions that the speed of mastering movements depends on them.

In the studied athletes, the distances between the finger triradii a and d were more than 47 mm, compared to the control group, where the distance ranged from 44 to 47 mm. And the distance between the finger triradius c and the main triradius t in track and field athletes exceeded 68 mm, while in the control group it was less than 68 mm. Thus, it can be concluded that the prediction of future athletes can be made by genetic markers of the distance between the finger triradii a and d, which should exceed 47 mm and the distance between the triradii c and t, which should exceed 68 mm (Fig 1).

The results indicate a possible connection between the anatomical characteristics of the fingers and the speed of mastering movements. Thus, in most athletes (59%), the distance between the triradii a and d of the fingers was 48 mm, and in students it was 46 mm. The distance between the triradius c of the finger and the main triradius t in most of the athletes (42%) exceeded 68 mm, while in the majority of the control group (44%) it was 65 mm. The results indicate a possible connection between the anatomical characteristics of the fingers and the speed of mastering movements.

So, the track and field athletes in the population of Ukraine were characterized by the features of the dermatoglyphic constitution: higher values of the deltoid index and the genetic markers of the distance between the finger triradii a and d.

DISCUSSION

Such researchers present fingerprints as a genetic marker due to their association with basic physical qualities and muscle fiber type [14]. Some researchers believe that physical qualities are determined both by

genetics and the environment, that is, by a combination of genetics and the environment [15]. For the detection of sports talent, dermatoglyphics has scientific evidence and importance, application and use in various contexts such as sports. Exercise is a powerful tool that affects a wide range of body parameters, and genetic factors will help guide the effects of exercise on the body [13, 16-18]. We agree with the authors (Fernandez et al., 2020) that dermatoglyphics is an effective method to characterize physical capabilities in the studied sports [19]. The aim of the study was to compare the dermatoglyphic markers of students and athletes, and to establish and practically substantiate the relationship between dermatoglyphic markers and the speed of reaction and type of temperament of track and field athletes.

It was determined that the most common dermatoglyphic markers of the control and main group of subjects is the loop. Genetic markers of track and field athletes will have a higher percentage of such finger patterns as radial loops and very little - archs. Athletes among the population of Ukraine tend to increase the number of radial loops ($p < 0.05$). Thus, our study confirms the research of other researchers [20-22].

Scientist M. Bogdanov proved the connection between dermatoglyphic signs and psycho-emotional components of a person's character. So, for example, people in whom arcs predominate among finger patterns are usually characterized by purposefulness, directness, frankness, but not very high intelligence [16]. The complete opposite of them are people with a predominant curly pattern. Such persons are characterized by a very high intellectual potential, but they usually cannot implement their ideas, which is associated with indecision and self-doubt. The golden mean between these two types is people with an average delta index, that is, those people whose dermatoglyphic pattern is represented mostly by loops.

Based on the analysis of our results, we can say that such a type of pattern as loops (L) prevails in all types of temperament in the main group. Using the results of dactyloscopy, it was established that the control group with a strong and balanced nervous system has more loops on the finger pattern, whorls in people with a strong and mobile nervous system, and archs in people with a weak nervous system. If we correlate the type of temperament and the finger pattern on ten fingers in percentage terms, the following turns out to be the case: choleric has more than 50% whorls; sanguine has more than 50% loops; phlegmatic usually has all loops; a melancholic has at least one arch, and the more there are, the weaker the nervous system, i.e. low efficiency.

According to the classification of character, tempera-

ment and behavior of a person according to skin patterns, it can be said that athletes of all types of temperament are dominated by such a finger pattern as a loops (L).

The value of the ATD angle and delta index for the palm was determined: we observe a tendency to decrease the ATD angle for the palm by 10% in track and field athletes among the population of Ukraine. The delta index in the control group has lower values (DI=9.5) than in the main group (DI=13.3). Thus, the delta index of track and field athletes among the population of Ukraine is 19% higher than that of students. This also indicates a tendency to increase the delta index in track and field athletes. The delta index provides an important basis for identifying athletics talent in children, because speed is essential for most sporting events. Many scientists recognize dermatoglyphics as key element in sports selection [16-21].

The relationship between dermatoglyphic markers and the prediction of performance in athletics was determined. Future track and field athletes can be predicted by genetic markers of the distance between the finger triradii a and d, which should exceed 47 mm and the distance between the triradii c and t, which should exceed 68 mm.

CONCLUSIONS

The research-descriptive nature made it possible to reveal the relationship between dermatoglyphic markers and the type of temperament of track and field athletes and students. It had a research character, since, at least in Ukraine, in the studied literature, there is no work that studies the relationship between dermatoglyphic markers and the type of temperament.

We established and practically substantiated the relationship between dermatoglyphic markers and reaction speed and temperament type of track and field athletes. By measuring the distances between the triradii a and d, c and t, we determined the relationship between the anatomical features of the fingers and the speed of mastering movements. Thus, for track and field athletes of the population of Ukraine, there were

characteristic features of the dermatoglyphic structure: higher values of the deltoid index and genetic markers of the distance between the triradii a and d of the fingers. The type of temperament is associated with such genetic markers as finger patterns, ATD angle for the palm, delta index (DI).

The predominant type of temperament in track and field athletes is sanguine. They refer specifically to the strong type of nervous system and its varieties, psychological properties, caused primarily by the strength, balance and mobility of nervous processes, are a necessary condition for achieving great success in sports. Among athletes, there is no such temperament as melancholic, it is a weak type of nervous system. Therefore, temperament affects the achievement of high results in athletics. Studying which temperament prevails in this type of sport will help athletes and coaches adjust the training process, better understand each other and, accordingly, achieve high results.

The track and field athletes in the population of Ukraine were characterized by the features of the dermatoglyphic constitution: higher values of the deltoid index and the genetic markers of the distance between the finger triradii a and d. Hereditary predisposition of the ATD angle was determined to be 37 ± 4.88 . Delta index of track and field athletes among the population of Ukraine is 19% higher than that of students. This also indicates a tendency to increase the delta index in athletes.

The study, provides a reference for future studies or for the detection and selection of talent for athletics. All the data that we reviewed confirm the opinion that the physical activity of a person can be influenced by the genetic markers presented in the article in speed sports. On the material of fingerprints and palm prints, and with the help of various statistical methods, the connections between dermatoglyphic signs and human temperament were studied. It should be noted that these are only general principles of determining a person's character using dermatoglyphics, because for a more accurate assessment of a person's character, it is necessary to take into account many other dermatoglyphic factors.

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ORIGINAL ARTICLE

HEPATITIS A. THE FEATURES OF DISEASE COURSE IN ADULTS

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ABSTRACT

The aim: To analyze the incidence of Hepatitis A in Ukraine and Poltava region and to study the clinical and epidemiological features of the course of Hepatitis A in adult patients.

Materials and methods: The course of HA in 96 hospitalized patients was analyzed. The diagnosis of HA was established on the basis of clinical and epidemiological data and confirmed by the results of laboratory studies (serological and molecular biological).

Results: In 2019, in the Poltava region, there was an increase in the incidence of Hepatitis A with a predominance among sick people of working age, among the urban population. This part of people aged from 60 to 75 years old constitutes 9.4%. This study showed that the waterway was the dominant way of HA transmission. The course of the disease in most hospitalized patients was typical and cyclic, with a predominance of a mixed variant of the pre-jaundice period and jaundice. One third of patients survey that they had fever, which persisted with jaundice.

Conclusions: The findings of this study indicates that the patients older than 40 years were more likely to have concomitant chronic pathology than younger patients, and Hepatitis A was more severe with the development of prolonged cholestasis, wave-like course and recurrence. In most patients under the age of 40, the course of Hepatitis A was mild, but splenomegaly and severe cytolytic syndrome were more common.

KEY WORDS: jaundice, hepatitis A, atypical course

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INTRODUCTION

Hepatitis A is recognized as being a widespread viral disease that occupies a leading position among acute infectious pathologies of the liver. According to the WHO (the acronym WHO stands for World Health Organization), about 1.4 million cases of hepatitis A (the acronym HA stands for hepatitis A) are registered annually in the world [1]. In Europe and the United States, the share of HA in the total incidence of viral hepatitis is from 10 to 30%, and in countries such as Asia, Africa, Latin America, endemic for this pathology is 70-80% [2, 3]. According to the WHO classification, Ukraine belongs to the regions with medium endemicity for HA, the criterion of which is IgG to HAV $\geq 50\%$ in persons under 15 years old and $<90\%$ up to 10 years old [4]. However, the level of dissemination of this virus in Ukraine is uneven. In recent years, there has been a downward trend in the HA infections in Ukraine. Nevertheless, the epidemic situation in the country remains tense. Thus, in 2019 there was an increase in morbidity by 6.5 times (19.03 per 100 thousand population) in Poltava region. It raised significantly in comparison to 2018 (2.92 per 100 thousand population) and it has exceeded the national on average 4.2 times (4.56 per 100 thousand population).

One of the leading factors determining the incidence rate of of HA is considered to be the sanitary and communal improvement of territories, and the main route of transmission of this infection is the waterway. According to the WHO, the most risky factors for HA infection are contact with a patient, the presence of unsatisfactory social conditions and lack of cleaned drinking water consuming, travel to a highly endemic region without being vaccinated against HA, homosexuality, parenteral drug use [4].

With the start of a full-scale war in Ukraine, the risks of outbreaks of infectious diseases have greatly increased [5], especially with the fecal-oral transmission mechanism. The lack of access for the people in the occupied territories to clean water, basic hygiene and medical care, crowding of people, in particular in shelters and temporary refuges, violation of the conditions for the burial of the dead plays a significant role in this process [6, 7]. Stress and malnutrition also increase the susceptibility of the population to infections. According to the Center for Public Health, for 6 months of 2022, the incidence of acute intestinal infections increased by 41.6% (9052 cases against 6390 in 2021) compared to the same period in 2021. Thus, the incidence of rotavirus infec-

tion increased by 62.3% (3533 cases vs 2176 in 2021), shigellosis by 49.2% (97 cases vs 65 in 2021). Although the number of cases of hepatitis A in 6 months of 2022 is 2 times lower than in the same period of 2021 (103 vs. 222 in 2021) [8], the presence of risk factors due to military action on the territory of Ukraine increases the likelihood of an outbreak of this infection.

According to scientific studies, about 90% of cases of HA have a benign course and end in recovery. However, 10-20% of patients recorded an atypical course of the disease with recurrence, prolonged cholestasis, fulminant liver failure, and induction of latent autoimmune hepatitis with a corresponding clinical picture in about 3% of patients [9-11]. The mortality rate from HA among children and adults under the age of 50 ranges from 0.3 to 0.6%, while in adults ≥ 50 years old it reaches almost 5.4% [12].

Recent studies have shown that HA has ceased to be a childhood infection, as the largest number of cases is registered in the adult population, and the patient's age is the most important predictor of the severity of the disease [13-15]. Therefore, given the current relevance of HA in Ukraine and the world, changes in the epidemiology of the disease, namely the increase in the average age of patients and, accordingly, the risk factors for adverse disease (chronic liver disease, HIV, etc.), in this study we examined the features of the current course of HA in adults.

THE AIM

To analyze the incidence of HA in Ukraine and Poltava region and to study the clinical and epidemiological features of the course of HA in adult patients.

MATERIALS AND METHODS

We analyzed the case histories (form No. 003/o «Medical record of an inpatient») of 96 patients with HA who were treated in the departments of the municipal enterprise «Poltava Regional Clinical Infectious Diseases Hospital of Poltava Regional Council» during 2019 were analyzed, among them the women – 53 (55.2%), men – 43 (44.8%), the patients aged from 18 to 71 years old (average 39.9 ± 1.5). The inclusion criteria for patients in the study were as follows: age over 18 years; presence of serum IgM to HAV, elevated alanine aminotransferase (ALT) activity. Patients younger than 18 years of age and those hospitalized with a diagnosis other than HAV were excluded.

The diagnosis of HA was established on the basis of clinical and epidemiological data and confirmed by the presence of IgM and IgG to HAV ("Vectogep" A-IgM,

JSC «Vector Best», Russia) and the absence of markers of other viral hepatitis (anti-HBcor (total), HBsAg, anti-HCV (total), which was determined by the immune fermentative analysis and (HBV DNA, HCV RNA) in the polymerase chain reaction ("Amplisens", the reagent kit, Russia).

Clinical severity of HA was determined by peak laboratory values. Severe HA was defined as a five-fold increase compared with the upper limit of normal for a particular sex of ALT or aspartate aminotransferase (AST) and the presence of at least one of the following criteria: serum bilirubin level $> 200 \mu\text{mol/L}$, hepatic encephalopathy or coagulopathy (international normalized ratio > 1.5). All other patients were defined as having mild HA. According to studies, patients over 40 years of age have an increased risk of developing HA-associated complications and mortality, so we analyzed the course of the disease in this category of patients compared to those under 40 years of age. All patients underwent clinical, laboratory, and instrumental examinations upon hospitalization and in the course of their hospitalization.

Analysis of the incidence of HA was performed according to the statistical reports of the Ministry of Health of Ukraine «Report on certain infectious and parasitic diseases» (reporting form № 2) for 2010-2019. Statistical analysis of the data was carried out by using the computer program "SPSS Statistics 17.0". The normality of the data distribution was checked using the Kolmogorov-Smirnov test. The mean (M) and standard error of the mean (m) or median (Me) with upper and lower quartiles (Q1-Q3) were used to determine the central tendency. In the case of normal distribution, the probability of differences in quantitative results for different groups of patients was determined using the Student's t-test, in the case of a distribution that differed from normal - the Mann-Whitney U-test, qualitative results - by analysing the contingency tables using the Fisher's exact test and the χ^2 criterion, depending on the prerequisites for the analysis.

RESULTS

It is established that during 2010-2018, an epidemic decline of HA with a morbidity rate 6.10 to 3.22 per 100 thousand population in 2010 and from 6.52 to 2.92 per 100 thousand population, respectively, in 2018 was reported as in Ukraine in general and in Poltava region in particular. In 2019, the incidence rates in the Poltava region and the city of Poltava exceeded the indicator for Ukraine (4.56 per 100 thousand population) by 6.5 and 11.2 times (19.03 and 51.19 per 100 thousand population, respectively) (Fig. 1).

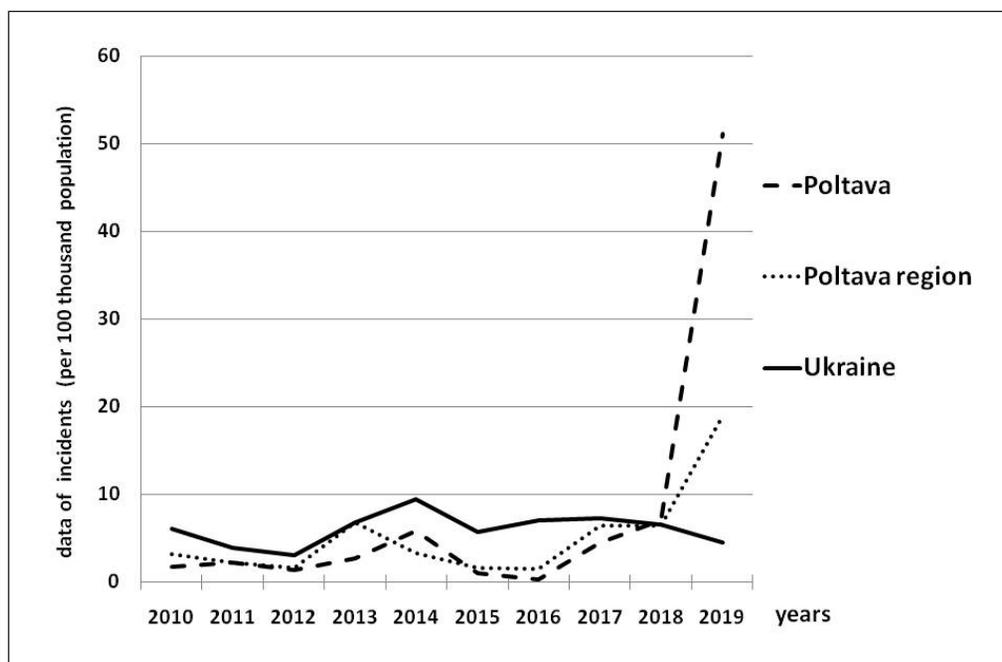


Fig. 1. Incidence of HA in Ukraine, Poltava region and Poltava (2010-2019)

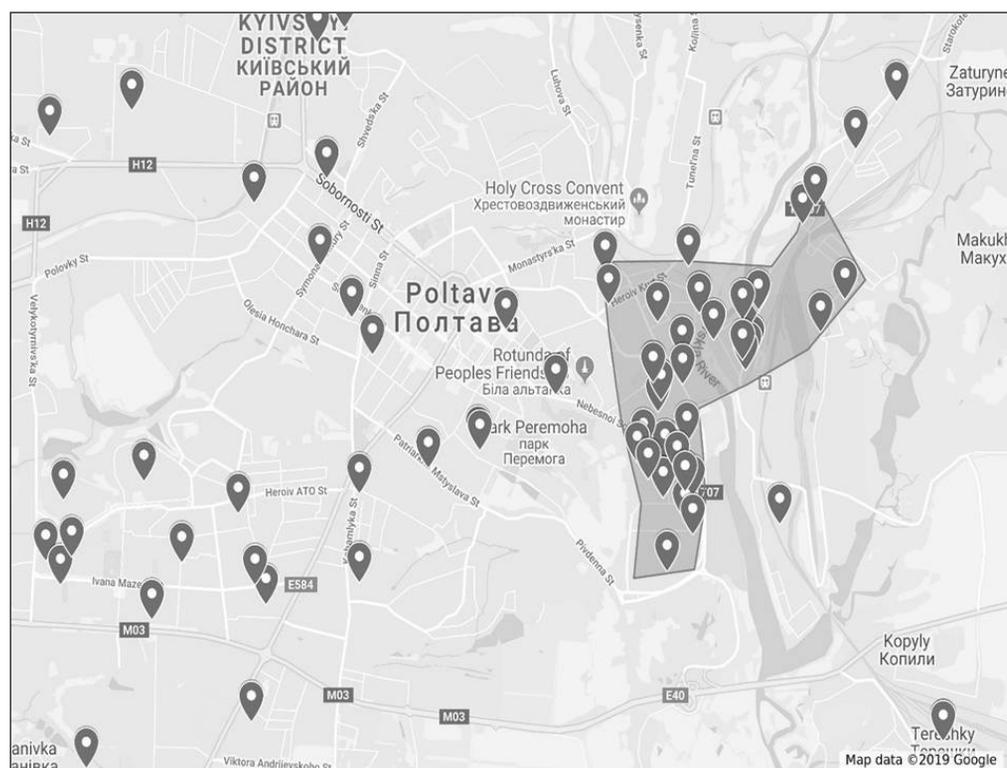


Fig. 2. Epidemic situation on HA in Poltava in 2019

The majority of cases (144 out of 265) which were registered in Poltava region are cases which fall for Poltava city itself, where the incidence rate increased by 7.2 times (51.19 in 2019 in comparison with the data of 2018, 7.08 per 100 thousand population). A detailed analysis of cases in Poltava showed that most patients lived in the Podol and Levada neighborhoods of the city, where there are some natural water reservoirs and one water intake, and the rest of the patients-respondents in other areas either contacted with them or worked in these areas (Fig. 2).

In 2019, among the patients hospitalized with HA the young and middle-aged patients comprises the biggest part (75.0%, n=72). The share of elderly people (from 60 to 75 years old) was 9.4% (n=9). According to the epidemiological history, 28 (29.2%) responded persons indicated that they had contacted with a HA infected patient, 12 (12.5%) responded patients had a swim in the open water reservoirs in Poltava region, rest of them had contacts abroad – 5 (5.2%), and finally, the others consumed unboiled water – 61 (63.5%) patients. The

Table I. Clinical and laboratory characteristics of patients with HA (n=96)

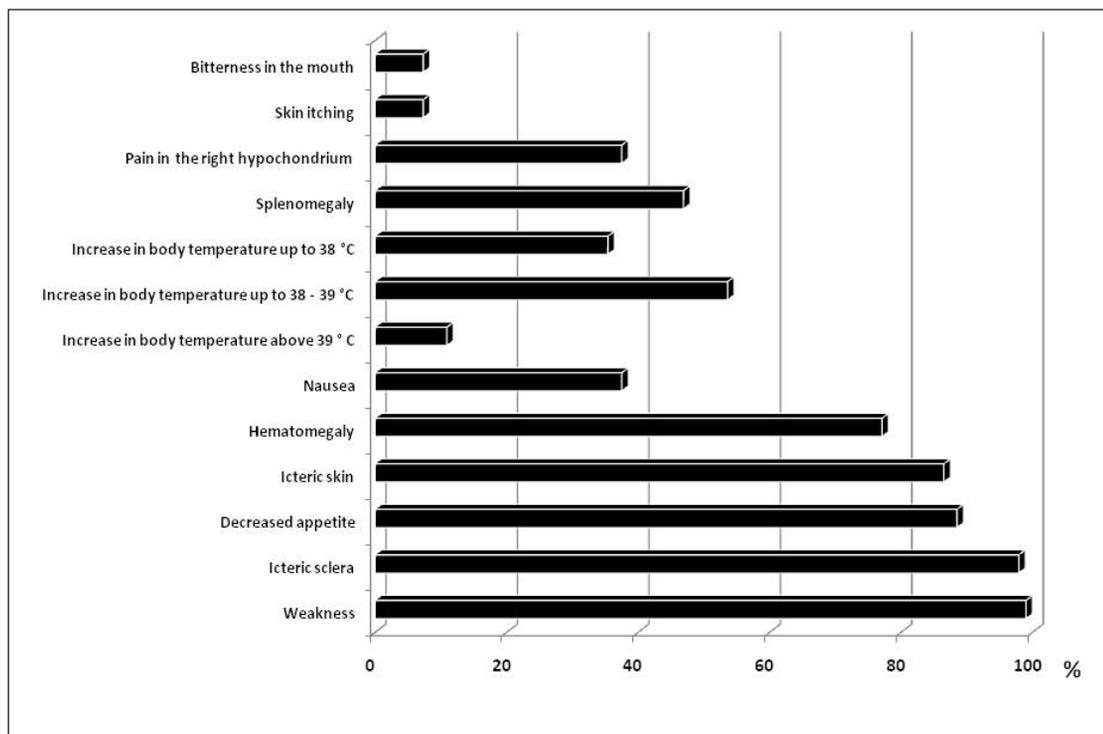
Characteristics	mild HA (n=84)	severe HA (n=12)	P
Gender:			
men	36 (42.9%)	7 (58.3%)	0.363
women	48 (57.1%)	5 (41.7%)	
Average age, years (M±m)	38,9 ± 1,54	47,4 ± 4,9	0.042
Duration of the jaundice period, days Me (Q1-Q3)	13 (10-17)	21.5 (14.5-38.0)	0.002
Duration of hospitalization, days Me (Q1-Q3)	17 (14,25-21,0)	30,5 (26-36,25)	0.007
Maximum serum bilirubin, µmol/l Me (Q1-Q3)	96,65 (65,73- 123,7)	257,05 (209,17- 282,17)	<0.01
Maximum activity of ALT, IU/l Me (Q1-Q3)	1218,0 (628,0-2159,25)	1779,0 (730,0-2729,0)	0.461

Note: p - level of significance obtained using Fisher's exact test, Student's and Mann-Whitney's tests, depending on the prerequisites of the analysis and the type of data

Table II. Clinical and laboratory characteristics of patients with HA younger and older than 40 years

Characteristics	Patients <40 years old (n=49)	Patients >40 years old (n=47)	P
Gender:			
men	22 (44.9%)	21 (44.7%)	0.983
women	27 (55.1%)	26 (55.3%)	
Course of HA:			
mild	45 (91,8%)	39 (83,0%)	0.228
severe	4 (8,2%)	8 (17,0%)	
Duration of the jaundice period, days Me (Q1-Q3)	13 (10-17)	14 (12-25)	0.049
Maximum serum bilirubin, µmol/l Me (Q1-Q3)	93.3 (59.3-137.25)	111.6 (88.8-177.8)	0.047
Maximum activity of ALT, IU/l Me (Q1-Q3)	1655 (751-2507.5)	946 (475-1779)	0.015
ESR increase, mm/h Me (Q1-Q3)	14 (28.6%)	23 (48.9%)	0.040

Note: p - level of significance obtained using Fisher's exact test, χ^2 and Mann-Whitney tests, depending on the prerequisites of the analysis and the type of data

**Fig. 3.** Frequency of the main clinical manifestations in patients with HA

general clinical examination revealed that 83 (86.5%) patients had chronic concomitant pathology, with a predominance of 74 (77.1%) diseases of the gastrointestinal tract (GI tract), which were more often represented by cholecystitis – in 34 (45.9%), gastroduodenitis – in 23 (31.1%), non-alcoholic steatohepatitis – in 11 (14.8%).

Jaundiced form of the disease was registered in 95 (99.0%), non- jaundice disease was registered in only 1 (1.0%) patient. According to the severity of HA, the subjects were distributed as follows: mild occurrence in 84 (87.5%), severe – in 12 (12.5%) patients. Patients were hospitalized from the 2nd to the 17th day from the onset of the disease, on average on the 7th day (interval 5-10), almost all were hospitalized after the onset of jaundice. The pre-jaundice period lasted from 1 to 15 days, the median for severe HA was 6.5 (5-9.75 days), and for mild HA - 5 (4-8) days. The mixed variant of the pre-jaundice period with signs of dyspeptic (91.7%), flu-like (77.1%) and astheno-vegetative (92.7%) syndromes prevailed over the others (95.8%, n=92). The first manifestations of the disease were the followings: general weakness (100.0%), loss of appetite (88.5%), pain in the right hypochondrium and nausea (37.5%). The patients 82 (85.4%) in the pre-jaundice period had a fever, which in a third (32.3%, n=31) of them persisted with the appearance of jaundice.

The frequency of the main clinical manifestations in the examined patients is presented in Fig. 3.

Comparison of clinical and laboratory characteristics in patients with mild and severe HA is presented in Table I. The mean age was higher in the group of patients with severe HA compared with mild HA (47.4 vs. 38.9 years, $p=0.042$). Patients with severe HA had a longer duration of the jaundice period and hospitalization, a higher median maximum bilirubin level as compared to patients with mild disease.

Since age over 40 years is a predictor of the severity of HA and worsens the prognosis, we analyzed the course of the disease in this category of patients (Table II). It was found that in patients over 40 years old, more often (87.2%, n=41), compared with younger ones (67.3%, n=33; $p=0.022$), chronic diseases of the gastrointestinal tract were recorded, which were represented by pancreatitis – 59.6%, cholecystitis (34.0 %) and non-alcoholic steatohepatitis – (34.0%) (in patients under 40 years old – 24.5% ($p<0.01$), 14.3% ($p=0.038$) and 10.2% ($p<0.001$) respectively). Severe HA was registered in patients older than 40 years 2 times more often, but without a significant difference when compared between groups ($p=0.228$). In patients older than 40 years, the duration of the icteric period was longer, the median maximum level of bilirubin was higher, and an increased erythrocyte sedimentation rate (ESR) was recorded more often. In contrast, in patients under 40 years of age, cytolysis rates were higher, in particular, the maximum of ALT activity was 1655 (751-2507.5

IU/l), in patients over 40 years of age – 946 (475-779) IU/l, $p=0.015$). According to the results of ultrasound examination of the abdominal organs in patients younger than 40 years, splenomegaly was detected more often – 57.1% (in patients older than 40 years – 36.2%, $p=0.042$).

The course of HA was typical and cyclic for 90 (93.7%) patients. A typical course of HA was registered in general – in 6 (6.3%) patients, only in persons older than 40 years (12.8%, n=47). It was represented by the development of long-term cholestasis – in 3.1%, wavy course – in 1.0% and relapses – 2.1% of patients.

DISCUSSION

HA is a cyclical disease characterized by epidemic outbreaks at certain intervals, which leads to a decrease in their clinicians' vigilance about this infection during periods of relative well-being. Our study shows that the relative epidemiological well-being of HA is imaginary, and is determined by large cycles disease characteristics as well as by a number of social factors. Therefore, the epidemic potential of HA with the evident threat of epidemic outbreaks today remains extremely significant. The increase in fatalities as a result of stratification of HA on other diseases affecting the liver and the possible development of autoimmune hepatitis after HA seem likely to prove that the problem of this infection is quite urgent for clinical investigations [14, 16].

At the present stage, HA has been acquiring new features different from the classical disease picture, which sometimes complicates the timely recognition of the disease. According to the scientific literature, this trend is especially observed in adult patients, who currently predominate among patients, and associate it with a premorbid background, which determines the severity of the disease [14, 15]. This is consistent with the results of our study, where 41 (87.2%) of 47 patients over 40 years of age were diagnosed with chronic comorbidities, most often gastrointestinal. In this age group of patients, HA was characterized by a more severe course with higher bilirubin levels, longer duration of the jaundice period and the development of atypical manifestations in 12.8% of hospitalized.

According to wide-ranging examination of the scientific literature, it is known that the frequency of atypical HA is from 1 to 20% (average about 7%) and its manifestations may be the development of relapses, prolonged cholestasis, acute liver failure and autoimmune hepatitis [16]. Much work on the issue mentioned above has been carried out, however there are still some data on the atypical course of HA which are need to be investigated. An increasing number of studies have found that the incidence of prolonged cholestasis in HA is less than 5%, and among the reasons for its development are coinfection with chronic hepatitis B, old age and high bilirubin [17]. The fulminant course of HA

with the development of acute liver failure in the developing countries is registered in 3.1–26% of patients over 50 years old. It is suggested that in addition to age, the causes for adverse disease may be provoked by genetic factors and excessive immune response involving CD8 lymphocytes and natural killer cells [18]. In 3% of patients, the HA virus induces latent autoimmune hepatitis I for 5 months after HAV infection [19, 20]. It was found that after infection in these patients there was a specific activation of CD4 T lymphocytes, as well as increased titers of antibodies specific to the asialoglycoprotein receptor of hepatocytes, which is considered to be the main target antigen in autoimmune hepatitis I. Relapses of HA, according to various studies, occur in 1.5–20% of patients and are defined as recurrent episodes of HA after an asymptomatic period of 4 to 10 weeks, with clinical and biochemical manifestations and the presence of HAV replication in the blood or stool by PCR. [21]. Scientific publications cite immune disorders as relapses, such as decreased ability to produce adequate levels of specific antibodies required for virus elimination, as demonstrated in studies in HIV-infected patients with HA [22].

Thus, the analysis of literature sources and our research results show that HA does not lose its relevance and topicality. Today there is a change in the epidemiology of HA with a predominance among sick adults with background somatic pathology, which can change and determine the course of the disease and complicate its diagnosis. Therefore, at the present stage, clinical variants, outcomes and long-term consequences of HA are of interest, and the most promising task is effective prevention of HA based on vaccination and its inclusion in the National Calendar of preventive vaccinations in Ukraine [23].

CONCLUSIONS

During 2010–2018, both in Ukraine and in Poltava region, HA was characterized by an epidemic decline, with incidence rates of 6.10 and 3.22 per 100 thousand people, respectively, in 2010 and 6.52 and 2.92 per 100 thousand people, respectively, in 2018.

In 2019, in Poltava region and Poltava city, there was a 6.5 and 11.2-fold increase in the incidence of HA (19.03 and 51.19 per 100 thousand people, respectively) with a predominance (75.0%, n=72) of people of working age and an increase in the proportion (53.4%, n=144) of the urban population.

According to the epidemiologic anamnesis, 76.0% (n=73) of hospitalized patients were diagnosed with waterborne transmission of HA.

The course of the disease in 90 (93.7%) of the examined patients was typical and cyclical with a predominance (95.8%, n=92) of a mixed variant of the prejaundice period and jaundice form (99.0%, n=95) of HA.

The majority (85.4%, n=82) of patients had fever in the prejaundice period, which in 32.3% (n=31) persisted with the onset of jaundice.

Patients over 40 years of age were more likely (87.2%, n=41) to have concomitant chronic gastrointestinal pathology compared to younger patients (67.3%, n=33; p=0.022). HA in this age group was characterized by a longer duration of the jaundice period (p=0.049), higher bilirubin levels (p=0.047) and, accordingly, a more severe course with the development of prolonged cholestasis in 3.1%, a wave-like course in 1.0% and relapses in 2.1% of patients.

Splenomegaly was more frequently detected in patients under 40 years of age (57.1%, 36.2% in patients over 40 years of age, p=0.042) and higher cytolysis rates were recorded (p=0.015).

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REX SHUNT IN CHILDREN: COULD DYSFUNCTION BE PROGNOSSED? A RETROSPECTIVE ANALYSIS

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ABSTRACT

The aim: To analyze retrospectively our experience of Rex shunt in children with symptomatic portal hypertension, its effect on hypersplenism regression and varices eradication, assess shunt survival and investigate risk factors, that could lead to shunt dysfunction and thrombosis.

Materials and methods: 24 children (16 males, 8 females), with portal hypertension included into the study. All surgeries were performed within single center in a period from January 2010 to March 2022. Follow up period was 6.75 ± 1.19 years.

Results: Age at diagnosis was 5.39 ± 0.64 years. 5 (20.8%) had umbilical catheter in anamnesis. 16 (66.7%) manifested bleeding episodes as the first sign of portal hypertension. 9 (37.5%) of children manifested severe hypersplenism. Age at Rex shunting was 7.5 ± 0.7 years. In 7 (31.8%) cases Rex shunt thrombosis occurred. 1 successful thrombectomy and 6 splenorenal shunting were performed. Kaplan-Meier analysis showed Rex shunt survival 0.670 (95%CI 0.420-0.831). Logistic regression model indicated thrombocytes count ($p=0.0423$) and cytopenia ($p=0.0272$) as factors that could influence shunt thrombosis. Follow-up group included 18 patients. Spleen volume regression became significant by 1 p/o year $p<0,05$, thrombocytes significant increase reached in 1 p/o months ($p<0.01$), varices involution was achieved by 1 p/o year ($p<0,001$).

Conclusions: Rex shunt effectiveness in study group was 70.9%, shunt survival assessed 0.670 (95%CI 0.420-0.831). Rex shunt was effective in bleeding prophylaxis in all patients of follow up group. Preoperative thrombocytes count ($p=0.0423$) and cytopenia ($p=0.0272$) were detected as factors that could influence shunt thrombosis, that is to be considered in RS preoperative period and require following studies.

KEY WORDS: Children, Portal Hypertension, Rex shunt, Shunt thrombosis, Extrahepatic portal vein obstruction

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INTRODUCTION

Current state methods of extrahepatic portal vein obstruction (EHPVO) surgical treatment in children are focused on two aims: to provide variceal bleedings prophylaxis and to restore the liver perfusion with splanchnic blood [1-4]. Since introduced first in 1992 for a posttransplant patient [5,6], Rex shunt (RS) restoring normal blood flow into the liver resolved at the same time secondary portal hypertension (PH) symptoms, such as recurrent bleedings and hypersplenism. However, it soon became clear that this surgery is feasible only in patients with favorable anatomy [7-9], a relatively small part of EHPVO pediatric patients. Not many authors dedicate their research to study the risk factors that could influence successful RS in pediatric patients [10,11], or factors that could affect shunt patency and therefore RS survival [4,12].

THE AIM

In this study we analyze retrospectively our experience of RS in EHPVO children with symptomatic PH, its effect

on hypersplenism regression and varices eradication, assess RS survival and investigate risk factors, that could lead to shunt dysfunction and thrombosis.

MATERIALS AND METHODS

Patients' data was collected from case-records retrospectively. Out of 475 children, who underwent surgical treatment for symptomatic portal hypertension, in 24 RS was considered feasible. All surgeries were performed within single center in a period from January 2010 to March 2022. The follow up period was assessed by September 2022, median 6.75 ± 1.19 years.

For all patients, the following criteria were analyzed: gender, age of disease debut, age at operation, basic symptoms and signs (bleeding episodes, thrombocytes count, spleen volume), complications, endoscopic examinations and procedures, the result of treatment, complications of treatment – shunt thrombosis occurrence.

Examination was as follows: CBC with thrombocytes count, thrombophilia panel, including protein C, protein

S, and antithrombin. Ultrasonography (US) in gray scale with spleen volume measurement (using the standard prolate ellipsoid formula: length \times width \times depth \times 0.523), color Doppler, and spectral Doppler tracings, which were performed as initial screening to each patient and at later stages of treatment and follow-up. US was also used for initial IJV assessment (patency and diameter). Endoscopy was performed in all patients (n=24) to assess the grade of esophageal varices and gastric mucosa, and to perform variceal band ligation when necessary. Contrast-enhanced CT was performed to all patients before surgery with additional 3D-modeling in recent cases, and on the first postoperative day. Initial neurologist consult was performed at admission to all patients of the study group.

Surgical procedure was performed according to the technique for RS described by authors [6]: laparotomy, Rex-recessus revision. If left portal vein branch and its outflow were considered patent and satisfactory, intraoperation portography was performed to visualize its intrahepatic branches. After superior mesenteric vein (SMV) was accessed. When SMV was identified as patent, venous autograft was harvested. Previously detected as better graft, left or right internal jugular vein (IJV) is anastomosed with left portal vein branch. And then, being passed through mesocolon "window", to SMV. In four cases gastric vein (GV) was appropriate for its length and patency, and therefore used as a "graft", preserving the natural IJVs: GV was dissected, ligated and after interposition anastomosed with left portal vein branch. Portal system pressure was measured intraoperatively before and after performing RS, to assess its decrease.

All patients received loading heparin dose of 5 units/kg in the moment of shunt forming and had continuous heparin infusion in maintenance dose of 10 units/kg/hour, to support the target ATTP level at 30 seconds and higher.

Data distributions were compared (for different surgical methods) using the paired Student's t-test or Wilcoxon criteria. The logistic regression model was built to identify factors that could influence the risk of shunt thrombosis. Kaplan-Meier estimator was used to assess shunt survival. Scheffe's method, Cruscal-Wallis multiple comparison and Dunn test were used to compare the follow-up results. Chi-square test was used to assess nonparametric data analysis for varices regression assessment. Statistical analysis was preformed using IBM SPSS for Windows version 24.0 (IBM Corp., Armonk, NY) and EZR (R-statistics). A *P*-value <0.05 was considered statistically significant.

The Committee on Clinical Investigation of Bogomolets National Medical University approved this

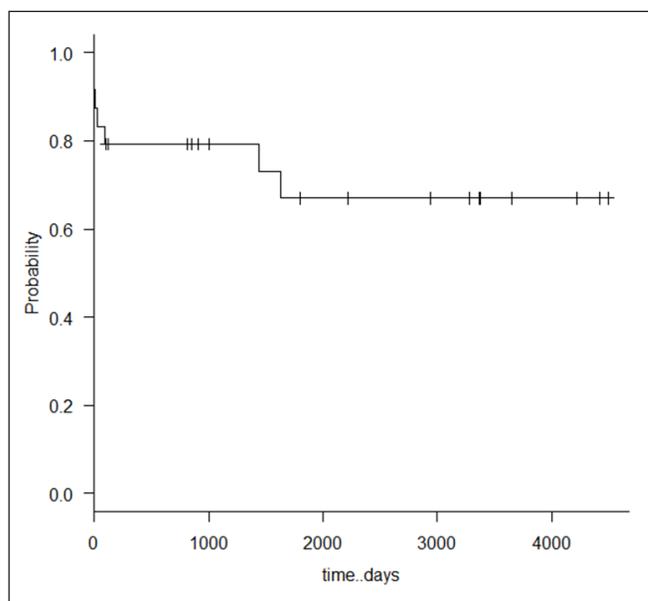


Fig. 1. Shunt survival (Kaplan-Meier curve) for RS (uniform line). RS survival 0.670 95%CI(0.420-0.831).

study (Protocol №141 27.01.2021). All the studies were conducted according to implemented guidelines in consideration of GCP-ICH and Declaration of Helsinki [13]. The written informed consent of all participants' parents/guardians was achieved.

RESULTS

Patients' characteristics are summarized in Table I. Male predominance (16 males and 8 females) can be seen. Mean age at diagnosis was 5.39 ± 0.64 years. 5 patients (20.8%) had umbilical catheter in anamnesis, in others EHPVO was considered as idiopathic. 3 children had surgeries in anamnesis: 1 - Sugiura procedure (complete portoazygos disconnection) for acute bleeding at the age of 1 y.o., 1 - Kasai procedure for biliary atresia at the age of 3 m.o., the same patient later underwent liver transplantation, 1- diagnostic laparotomy at the rural hospital. 16 (66.7%) patients manifested bleeding episodes as the first sign of PH, 5 of them were admitted with signs of acute bleeding. Others (n=8) were directed to examination for accidentally detected splenomegaly either anemia. According to Endoscopy data, only 1 of 24 patients had initial grade I esophageal varices. Other 23 had grade II – grade III varices (Figure 5-a). In 5 cases surgery was urgent. By the time of surgery all patients had splenomegaly with median spleen volume of 453 cm^3 (95%CI 353-503) and thrombocytopenia with mean thrombocytes count of 62.6 ± 6.2 . 9 (37.5%) of children developed severe hypersplenism, with cytopenia: leucocytes count less than $1.5 \cdot 10^9/\text{l}$, erythrocytes count less than $1.8 \cdot 10^{12}/\text{l}$,

Table I. Characteristics of study group patients

Characteristic	Total	Percent
Gender		
Male	16	66.7%
Female	8	33.3%
Aethiology		
Umbilical catheter	5	20.8%
Idiopathic	19	79.2%
Anamnesis at admission		
Previous surgeries	3	12.5%
Bleeding episodes	16	66.7%
Clinical presentation		
Splénomegaly	24	100%
Severe hypersplenism with cytopenia*	9	37.5%
...Isolated thrombocytopenia	2	8.3%
Isolated anemia without bleeding in anamnesis	3	12.5%
Ascites	2	8.3%
Esophageal varices at admission		
Grade I	1	4.1%
Grade II	6	25%
...Grade III	17	70.9%

* Leucocytes less than $1.5 \cdot 10^9/l$, erythrocytes less than $1.8 \cdot 10^{12}/l$ regardless of acute or anamnestic bleeding episodes

Table II. Risk factors that were included into the logistic regression model of RS thrombosis risk

Risk factors	OR (95% CI)	p-value
Preoperative acute bleeding episode	0.107 (0.000342000 - 33.50)	0.446
Bleeding episodes in anamnesis	3.4300 (0.14200000 - 82.50)	0.448
Learning curve	0.908 (0.69800000 - 1.18)	0.476
Umbilical catheter in anamnesis	2.550 (0.04430000 - 147.0)	0.65
Thrombocytes count	1.030000 (1.00000000 - 1.060)	0.0349
Preoperative portal system pressure	0.9840 (0.94800000 - 1.02)	0.387
Cytopenia ^a	141.000000 (1.63000000 - 12200.0)	0.0297

^a Leucocytes less than $1.5 \cdot 10^9/l$, erythrocytes less than $1.8 \cdot 10^{12}/l$ regardless of acute or anamnestic bleeding episodes

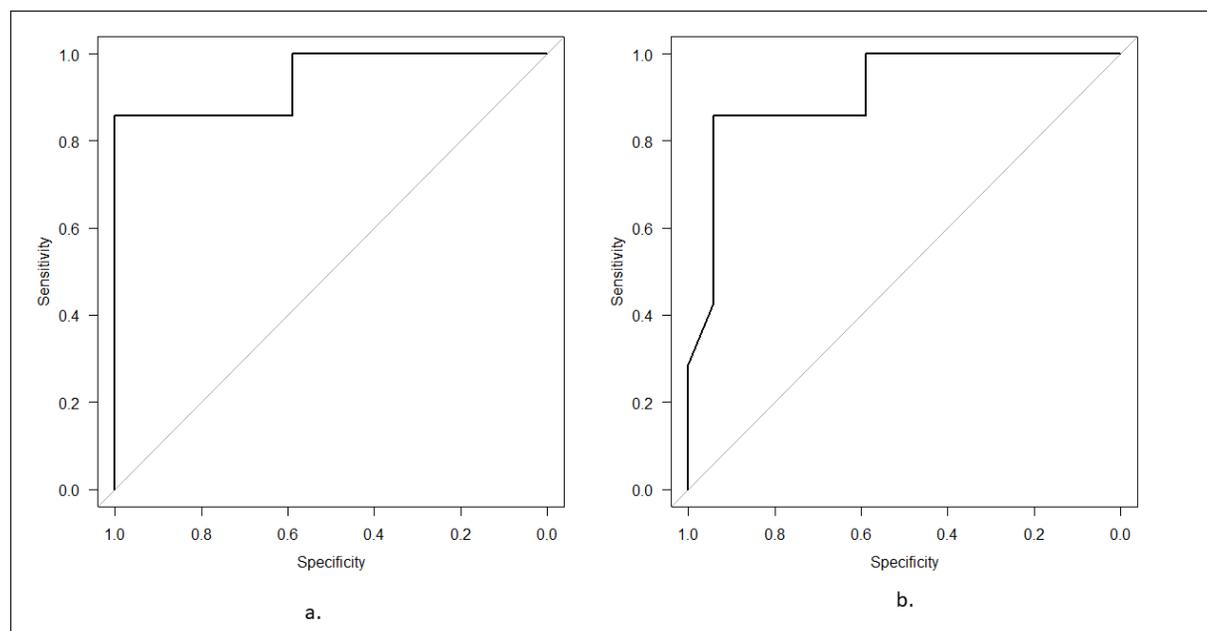


Fig. 2. a. ROC-curve of 7-factor logistic regression model of RS thrombosis occurrence risks. AUC= 0.941 (95% CI 0.821 – 1). b. Final 2-factors logistic regression model of RS thrombosis occurrence risks AUC= 0.912 (95% CI 0.78 – 1).

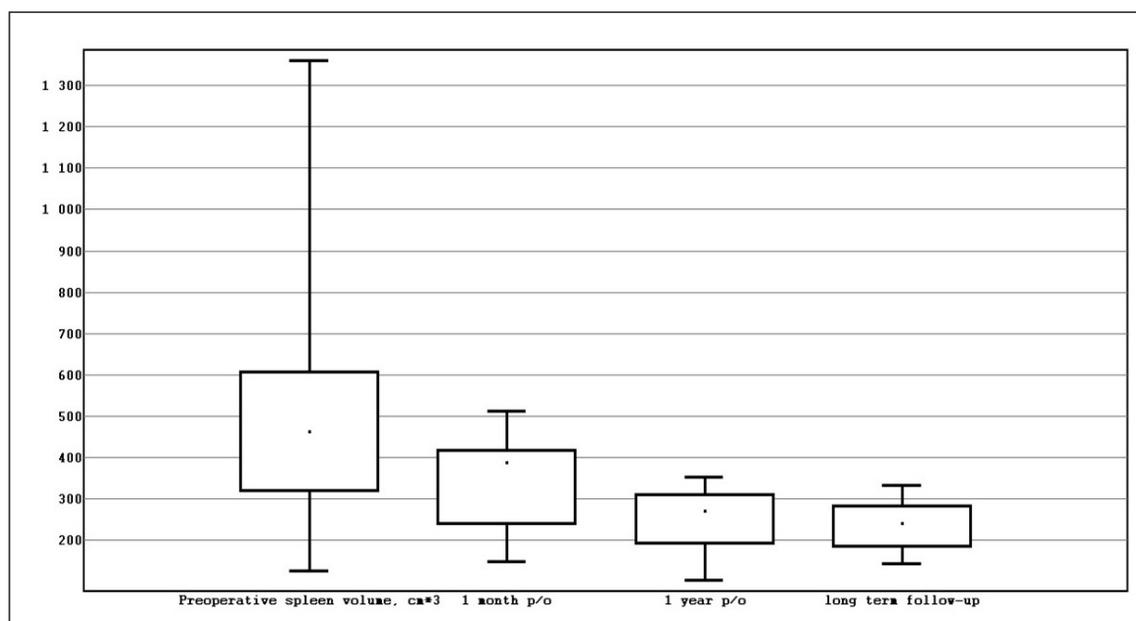


Fig. 3. Spleen volume regression graphic. Preoperative median volume was 453 cm³ (95%CI 353-503), 1 month after MPS 388,5 cm³ (95%CI 239,5-418,5), 1 year p/o 271,5 cm³ (95%CI 191,5-310), and 242 cm³ (95%CI 183-283,5) in long term follow-up.

regardless of current or previous acute bleeding in anamnesis. In 3 isolated anemia (hemoglobin level lower than 70 g/l) without registered bleeding episodes in anamnesis, and 2 had isolated thrombocytopenia with low but normal leucocytes and erythrocytes counts. In 5 (22.8%) patients acute variceal bleeding short-term conservative treatment by means of octreotide 2mg/kg/hour in continuous intravenous infusion and RBC transfusion at the rate of 5 ml/kg. 3 (12.5%) required urgent band ligation procedure.

Mean age at RS was 7.5±0.7 years. Median operation time for RS was 320 (95%CI 280-357) minutes, shorter in cases when GV interposition was used to redirect blood into the liver. No perioperative complications occurred. Median initial pressure in portal blood system was 347.5 mmHg (95%CI 320-360). After RS in both modifications portal blood system pressure was 212.5 mmHg, (95%CI 200-230)mmHg. T-Wilcoxon criteria showed significant decreasing (p<0.001).

In 7 (31.8%) cases shunt thrombosis occurred. Early thrombosis occurred in 3 cases. On the 1 p/o day in a patient in which later thrombophilia was confirmed: enhanced CT showed the absence of the blood flow in the shunt. Relaparotomy was performed, the graft was found filled with thrombotic masses for all its length, graft patency was impaired, and therefore blockage of vessel anastomosis was recognized, and venous graft was removed. Splenorenal shunting was performed. On the p/o day 2, confirmed by repeated Doppler US and CT. In this patient thrombectomy was successfully performed with following uneventful postoperative course. The absence of blood flow in the shunt was revealed

on p/o day 6 by repeated Doppler US and following CT in the third patient with early RS thrombosis. 1 patient manifested ascites on p/o day 27. The rest 3 patients in which shunt thrombosis occurred in p/o months 3, 48, and 68 correspondingly, manifested bleeding episodes. All had developed portal hypertension symptoms, such as high-grade varices, splenomegaly, and thrombocytopenia recurrence. Splenorenal shunting was performed in latter four described cases.

Kaplan-Meier survival analysis was performed to estimate RS survival. Median overall shunt survival was not reached by the patients of the study group (Fig 1).

For multifactorial analysis logistic regression model was constructed (Figure 2a) to identify factors that could prognose the risk of RS thrombosis. Factors investigated are represented in Table II.

Stepwise method revealed two factors were revealed that are connected to RS thrombosis risks: "Thrombocytes count" and "cytopenia". Based on these two factors logistic prognosing model was constructed (Figure 2b).

Follow-up group included 18 patients, 17, whose postoperative course and follow-up were not complicated and 1, who successfully underwent thrombectomy and reshunting. No recurrent bleeding episodes were registered. All patients were seen first one month after surgery to undergo a Doppler US and CBC with Thrombocytes count. Later laboratory tests and Doppler US took place once in two months, and from half a year after surgery – once in 6 months, from a second year after surgery – once a year. First Endoscopy was performed in 6 months after surgery, later – in every annual follow-up visit. 6 (33.3%) patients of the follow

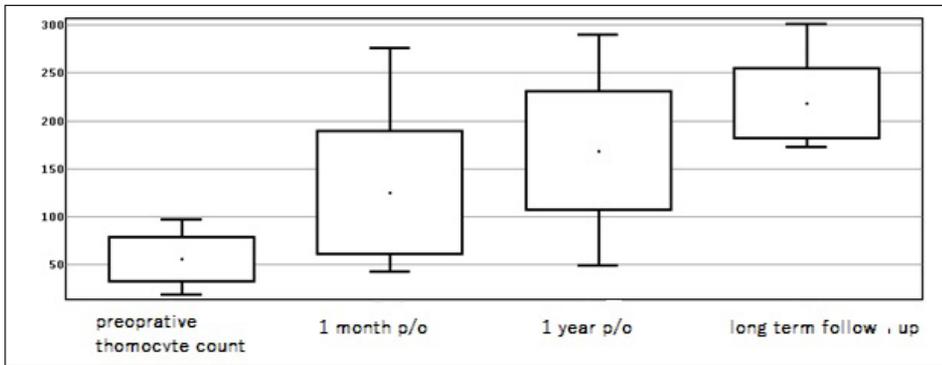


Fig. 4. Evolution of thrombocytes. Initial preoperative mean thrombocytes count was 55.4 ± 5.8 , 1 month after MPS 125.1 ± 16.11 , 1 year p/o 168.6 ± 15.63 , and 218 ± 9.24 in long term follow-up.

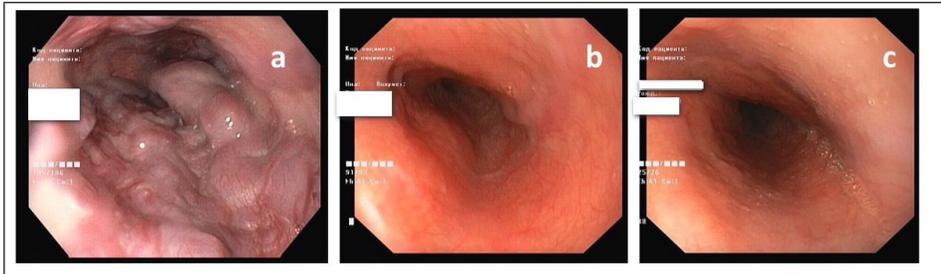


Fig. 5. Endoscopic appearance of the same patient within the course of treatment and follow up.

a. Preoperative endoscopic appearance - varices grade 3.

b. 1 year p/o – varices grade 1.

c. long term follow-up - varices grade 0.

up group reached age over 18 years by the end of assessed follow-up period. One of adult patients was at 18 weeks pregnant by the moment of study completion, the course of pregnancy was uneventful.

Dunn's test was used for multiple-comparison procedure of spleen volume regression assessment. Spleen volume regression was not significant by the first p/o month $p > 0.05$, became significant by 1 p/o year $p < 0.05$, and in long term follow-up $p < 0.001$ (Fig 3).

Preoperatively all patients manifested thrombocytopenia. Thrombocytes count was analyzed by means of Sheffe's multiple analysis test, that showed thrombocytes significant increase in 1 months, 1 year and in long term follow-up after RS ($p < 0.01$ in all cases). In long term follow up Thrombocytes count normalized in all patients. Results are represented on Fig 4.

Before RS 17(70.9%) patients had grade 3 varices, and 6(25%) – grade 2 varices. Two of them underwent preoperative varices band ligation procedure preoperatively. Endoscopy was repeated 1 year after RS and revealed grade 1 varices in 8 (50%) patients, and grade 2 in all other children of the group. In long term follow-up 6(37.5%) patients had grade 0, 9(56.3%) patients had grade 1, and 1(6.2%) patient had longstanding varices grade 2, that required 2 procedures of endoscopic ligation. Two patients of the follow up group have not reached the long-term period yet. Chi-square test was used to analyze varices involution and revealed the difference $p < 0.001$. Endoscopic appearance is represented on Figure 5. It represents initial preoperative endoscopic appearance (Figure 5a), varices eradication to safe grade 1 (Figure 5b) which followed in one year after RS, and total eradication in long-term follow-up,

which was 2.5 years for this specific patient on the represented image (Figure 5c).

DISCUSSION

Despite endoscopic bend ligation success in variceal bleeding primary prophylaxis [14-16], all portal hypertension symptoms, such as hypersplenism, cytopenia, encephalopathy, and growth impairment, only surgical treatment, and RS particularly, can resolve [2,3,17,18]

Some authors name RS and "ideal", the only physiological shunt, which provides the liver perfusion restoration, contemporaneously eliminating all portal hypertension signs [2, 3, 12, 16, 19]. Since the very first publication in virtue of further research RS was considered a "golden standard" for treatment of children with EHPVO [20], however noting patients should have favorable anatomy. Some authors emphasize RS is to be performed in asymptomatic patients who were already diagnosed EHPVO and in which visualization showed intrahepatic portal vein branches patency [1, 2, 7, 21, 22]. Other authors [4, 23] advice to perform RS in patients with high grade varices and bleeding risks, considering this surgical procedure the best bleeding prophylaxis method. Planned RS would provide the physiological development in a child with restored portal blood flow against the background of pathologic portal hypertension symptoms. Moreover, some authors mention encephalopathy resolution [4, 22, 24], growth acceleration [19], hepatopulmonary syndrome recovery, and vanishing of adenomas and focal nodular hyperplasia of the liver tissue [4,7] in patients who underwent RS. In a study where children with EHPVO

who were treated by means of RS it is mentioned that RS is effective in physical development improvement and optimizing of liver metabolic function [19, 24].

All patients from the study group were initially consulted by the neurologist. Encephalopathy was registered in none, therefore was not followed further. Growth impairment was not significant among children of the study group, and was not the purpose of our study, therefore, not assessed within it.

5 (20,8%) children of our group were admitted with acute bleeding as the first sign of PH. All were considered healthy before this initial hospitalization. We consider unique compensatory reserve of child's organism, absence of regular physical examination culture, and low awareness of primary care pediatricians to be the key causes of late EHPVO with symptomatic PH identification. Surgical treatment postponement since the settled diagnosis, that was 1.5 years (3 months – 15 years) in the children of our study group was determined by young age and initial visualization results, that were considered as not appropriate for any type of shunting (n=3).

We consider variceal band ligation to be effective as a primary bleeding prophylaxis, as it is already mentioned in our earlier publications [14,15]. Band ligation is widely used in our clinic since 2017, therefore all patients treated before 2017, which were included into the study group, were prescribed either conservative therapy, or urgent surgery when admitted with acute bleeding.

It was already shown in early studies that US shows signs of patency of Rex-zone only in 63% patients [25]. In the last two decades CT improved intrahepatic portal branches visualization significantly, however authors mention that in patients with large cavernoma CT or MRI results are not reliable sometimes, as hypodynamic circulation of intrahepatic portal branches and Rex-zone is visualized [9,20]. Bambini D.A. et al [8] in his study mentioned, that in a part of patients of his group, whose visualization results showed no patency in Rex-zone, RS was eventually feasible. Other authors consider direct intraoperative visualization to be the best way to detect left portal branch patency [8, 9]. We used all the mentioned techniques in patients of our study group.

RS with IJV allograft surgical technique corresponded to one published by De Ville De Goyet et al. in the beginning of 1990th [1, 6] and further detailed descriptions [21,23]. Except this technique, it was also proposed to reconnect left intrahepatic portal branch with splanchnic blood communicants by means of large saphenous graft [12, 26], or by means of gastric, spleen, inferior mesenteric vein grafts, or large cavernoma branch interposition [1, 27].

In our study group it was possible to perform RS with GV interposition in four cases, which allowed to preserve patients natural IJVs. 1 out of 7 thrombosis occurred among these patients.

The initial idea of multifactorial analysis logistic regression model construction was to prove umbilical catheter in anamnesis is not the factor that could influence the risk of mesoportal shunt thrombosis in children of our study group. Some authors mention umbilical catheter as unfavorable factor for following RS success [23], or even state it as a contradiction [4]. We had 5(20.8%) patients with umbilical catheter in anamnesis in our group, only one of them developed shunt thrombosis, the only child in which thrombophilia was confirmed. Other study revealed anticoagulation therapy regimen and blood transfusion as factors that could influence the risk of mesoportal shunt thrombosis [10]. As all the patients from study group received the same anticoagulation therapy, this factor was not included into the model. In other study [12] authors detected low body mass study and preoperative thrombocytes count as factors which could influence mesoportal shunt thrombosis occurrence. Bleeding episodes in anamnesis ($p=0.448$) either acute bleeding within a week in preoperative period ($p=0.446$) showed no significance as RS thrombosis risk factors. It is well known RS is a procedure for a surgeon of expertise, considering the fact all surgeries were performed by one team, it was suggested the learning curve could matter, but it didn't either ($p=0.476$). According to multivariate analysis in our study preoperative thrombocytes count ($p=0.0349$) and cytopenia ($p=0.0297$) were identified as risk factors for RS thrombosis development, which should be, to our thought, considered in the preoperative period. We have not assessed growth impairment; therefore, the body mass was not included into factors investigated.

Chin A.C. showed previous portal hypertension surgeries have deleterious effects on RS results [11]. Three of patients from our group had previous surgeries, and all three had uneventful postoperative RS course.

According to literature, shunt occlusion occurs in 1.6-16.4% cases [3] and was not observed in patients of our group. In his study Zhang Z. showed that according to currently published studies shunt thrombosis occurs in 8-40% of described groups [3]. There were 7 episodes of RS thrombosis registered in our group, which is 29.1%. Therefore, RS effectiveness in our group is, 70.9%, with RS survival assessed as 0.670.

The main role in RS thrombosis detection belongs to CT [20]. Enhance CT was performed in all children with RS thrombosis to confirm the diagnosis.

Discussing the surgical tactics in cases of RS thrombosis, Zhang, J.-S. Et al. in their study showed splenorenal shunting was a better treatment after bypass failure,

with faster recovery and symptoms relief than those who underwent re-shunting [27]. At the same time, it must be mentioned that children of their group developed RS thrombosis in long-term follow-up period with bleeding episodes in most cases. In our group successful RS reshunting was performed on the second postoperative day, and this patient was included into follow-up group according to his following uneventful postoperative period. All the other children with RS failure underwent splenorenal shunting, that corresponds to the results presented in the mentioned study.

Most authors show in their studies thrombocytes count restoration and significant decrease or normalization of spleen volume within 6-12 months after RS. [1,8,10,12]. In our study thrombocytes count significantly increased by the first postoperative year, together with spleen size and volume significant decrease, that correlates with data provided in literature sources.

CONCLUSIONS

RS is a golden standard surgical treatment of children with symptomatic PH caused by EHPVO, that restores the splanchnic blood flow into the liver and resolves portal hypertension symptoms, such as, esophageal varices with bleeding episodes, splenomegaly, and hypersplenism. Different surgical techniques that are used to perform RS provide wider arsenal of possibilities to restore the splanchnic blood flow into the liver in children with PH. RS effectiveness in the study group was 70.9%. RS gave excellent result in thrombocytes count increase, varices eradication, and normalization of spleen volume, therefore was effective in bleeding prophylaxis. RS thrombosis occurred in 7 (29.1%) cases. Preoperative thrombocytes count ($p=0.0423$) and cytopenia ($p=0.0272$) were detected as factors that could influence shunt thrombosis, that is to be considered in RS preoperative period and require following studies.

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OTOTOXICITY OF ANTIMYCOBACTERIAL THERAPY: MANIFESTATIONS, MECHANISMS OF MANAGEMENT AND CONTROL

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ABSTRACT

The aim: To study the nature and incidence of hearing loss related to tuberculosis (TB) or resulting from antimycobacterial therapy, and its impact on treatment outcomes in patients with multidrug-resistant TB (MDR-TB).

Materials and methods: An analysis of reports on adverse reactions, medical records and electronic database of the register of TB patients was made. The pathogen was microbiologically verified in all the patients. Patients underwent clinical and laboratory, instrumental, microbiological (BACTEC), molecular genetic (Xpert® MTB/RIF® Ultra, Xpert® MTB/XDR, GenoType® MTBDRplus/sI) examinations. To prevent the development of complications and to control adverse effects, alongside with the determination of the corrected QT interval, visual acuity, and color vision, brief peripheral neuropathy screen and audiometry were performed.

Results: During MDR-TB treatment with aminoglycosides, therapy was more commonly interrupted during the second episode of therapy ($p=0,051$), while treatment failure, longer treatment duration, and hearing impairment were almost equally observed in both groups ($p=0,431$, $p=0,432$, $p=0,69$). Treatment success was more commonly observed among patients receiving the first course of therapy. Some patients undergoing repeated antimycobacterial therapy were transferred to palliative care ($p=0,13$). The short-term treatment regimen effectively prevented ototoxicity.

Conclusions: Novel antimycobacterial agents and short-term TB treatment regimens increased patient compliance with treatment and reduced the incidence of certain adverse effects due to their monitoring and prevention. Due to the transition to mainly drug therapy, adverse effects such as ototoxicity were completely eliminated. This was due to personalized treatment selection, its monitoring, and assessing the outcomes.

KEY WORDS: multidrug-resistant tuberculosis, ototoxicity, treatment efficacy, adverse effect

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INTRODUCTION

A thousand-year history of tuberculosis (TB) is the history of mankind's struggle against the disease. This war for health continues today. Due to the discovery of antibiotics to which mycobacteria are sensitive, a strong arsenal against infection has been obtained. However, an extremely urgent concern which affects treatment efficacy and disrupts its possibilities - antibiotic resistance and adverse effects, has arisen [1,2]. The emergence of multidrug-resistant mycobacterial strains and the development of adverse effects result in treatment interruptions or therapy termination due to patient's refusal and the development of severe destructive TB forms. TB treatment regimens with injectable agents, specifically aminoglycosides, result in the development of ototoxic side effects which are irreversible [3-6]. The 8-month intensive phase of MDR-TB treatment with daily intramuscular administration of aminoglycosides, that was the standard chemotherapy for TB, did not change for a very long time [1-13], which

often resulted in toxic damage to the ear – sensorineural hearing loss (SHL).

Factors that potentiate each other and contribute to the clinical presentation of the disease such as the toxic effect of mycobacterial by-products, the specific effect of aminoglycosides on the vestibulocochlear analyzer, the general toxic effect of antimycobacterial therapy, impaired blood rheology and microcirculation, allergic sensitization to antibiotics, are involved in the pathogenesis [3,5]. Other risk factors include concomitant ear diseases and disorders, head injuries, cerebrovascular accidents, myositis, etc. The mechanisms involved in ototoxicity are as follows: the competition between calcium and magnesium ions on the cell surface; an active energy-dependent transport of aminoglycosides into the cell; irreversible binding of antibiotics to phosphatidylinositol phosphate (PIP2), a physiologically important phospholipid of the cellular membrane which is involved in transferring second messenger signaling that causes long-term damage to recep-

tor cells. As a result, damaged ribosomes, altered protein synthesis, impaired oxygen metabolism in the inner ear are seen. The great challenge we face is the impossibility to eliminate the main causative agent of ototoxicity since aminoglycosides, as one of the first-line drugs for MDR-TB, should be used for a long time, that, in its turn, increases the risk of complications [1,5-7]. In addition, the approach to correcting adverse effects is complicated by the negative potentiation of antimycobacterial therapy ototoxicity by vascular agents included in the protocols for providing medical care 'Otolaryngology'.

A new era has set ambitious goals and built strategies of their implementation – less than 1 case per million population and no TB deaths by 2050. Novel roadmaps for TB elimination include the latest rapid diagnostic methods (Xpert® MTB/RIF® Ultra, Xpert® MTB/XDR, GenoType® MTBDRplus/sl, whole-genome sequencing), a wide range of modern antimycobacterial drugs (linezolid, bedaquiline, delamanid, pretomanid), relevant, short-term drug regimens, monitoring and prevention of adverse effects [4,8,10,11].

The new paradigm is the activation of the maximum efforts of the entire community, including medicine, for the control and elimination of TB. The principles of preventing and controlling infectious diseases should not be neglected. If one almost forgot about an infectious disease, it doesn't mean it was eliminated and disappeared. No, it persists, but constant surveillance, prevention, timely diagnosis, and high-quality treatment can protect us against TB, an insidious disease humanity has been battling for many centuries.

THE AIM

The paper analyzed the incidence of ototoxic responses to anti-TB drugs and treatment outcomes in patients with MDR-TB depending on the chosen treatment regimen. A case report of ineffective treatment with the transition from curative to palliative care in a patient who developed pronounced ototoxic changes is presented. The ability to completely eliminate ototoxic changes and minimize other side effects of antimycobacterial therapy, provided that short-term therapy regimens are used.

MATERIALS AND METHODS

An analysis of 374 reports on adverse reactions, medical records and electronic database of the register of patients treated at a communal non-profit enterprise "Ivano-Frankivsk Regional Phthisiopulmonology Center of Ivano-Frankivsk Regional Council" between 2017-2019 and 2020-2022 was made. The division into groups took place according to the frequency of episodes of treatment

for tuberculosis. The first group is the primary treatment case, the second group is the repeated treatment case. TB was microbiologically verified in all the patients. According to the order of Ministry of Health of Ukraine (MoH) No. 420 dated September 14, 2014 "Unified Clinical Protocol of Primary, Secondary (Specialized), and Tertiary (Highly Specialized) Medical Care Tuberculosis" and the order of the MoH No. 530 dated February 25, 2020 and its edition No. 2161 dated October 6, 2021 "On Approval of Healthcare Standards in Tuberculosis", all the patients underwent mandatory laboratory, instrumental (X-ray, computed tomography, spirometry, fibrobronchoscopy, lung ultrasound, abdominal ultrasound, audiometry), molecular genetic (Xpert® MTB/RIF® Ultra, Xpert® MTB/XDR, GenoType® MTBDRplus/sl) examinations, sputum sample cytology, and phenotyping (BACTEC system, solid Lowenstein-Jensen medium) [11]. Adverse effects were monitored during treatment and according to the scheme developed on the basis of patient categories: peripheral neuropathy (PN) was graded using the scale for subjective PN assessment; audiometry was applied to identify impaired hearing; the corrected QT interval was determined; visual acuity and color vision were measured by the Ishihara color test; serum levels of bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) as indicators of hepatotoxicity and creatinine clearance levels as markers of nephrotoxicity were measured; complete blood count (CBC) was done to check for myelosuppression [8,10]. The data obtained were statistically processed using the Microsoft Excel package of statistical functions. The significance of the difference was determined by the Fisher's F-test for parametric data. A test statistic is about the ratio of sample variances.

RESULTS

An analysis of reports on adverse events during 2017-2019 showed that among 193 documented cases, 55 (28.5%) adverse effects followed treatment of drug-sensitive TB and 138 (71.5%) side effects developed in MDR-TB. The treatment regimen for MDR-TB included pyrazinamide (Z), levofloxacin (Lfx), aminoglycosides – kanamycin (Km) and capreomycin (Cp), ethionamide (Eto) or protionamide (Pt), cycloserine (CS), para-aminosalicylic acid (PAS) for 20 months, with the 8-month intensive phase involving once-daily intramuscular injections of aminoglycosides. During MDR-TB treatment, there were 29 (21.0%) cases of ototoxicity, including SHL, hearing loss, and its coexistence with vestibular disorders (dizziness, balance problems, unsteady gait). The rate of onset, the intensity of manifestations, and the effect on treatment outcomes depended on the number of treatment episodes (Table I).

Table I. Treatment efficacy in patients with ototoxic manifestations who received anti-tuberculosis drugs during 2017-2019, %

Treatment efficacy	Treatment episode I, n=10	Treatment episode II, n=19	Fisher's exact test, p
Treatment interruption	4 (40.0)	15 (78.9)	=0,051
Treatment failure	2 (20.0)	7 (36.8)	=0,431
Longer treatment duration	5 (50.0)	13 (68.4)	=0,432
Treatment success	8 (80.0)	7 (36.8)	=0,05
Hearing abnormality	5 (50.0)	12 (63.2)	=0,69
Palliative treatment	0 (0.0)	5 (13.7)	=0,13

Table II. Treatment efficacy in patients with ototoxic manifestations who received anti-tuberculosis drugs during 2020-2022, %

Treatment efficacy	Treatment episode I, n=2	Treatment episode II, n=2
Treatment interruption	0	0
Treatment failure	0	0
Longer treatment duration	1 (50,0)	2 (100)
Treatment success	2 (100)	2 (100)
Hearing abnormality	0	1(50,0)
Palliative treatment	0	0

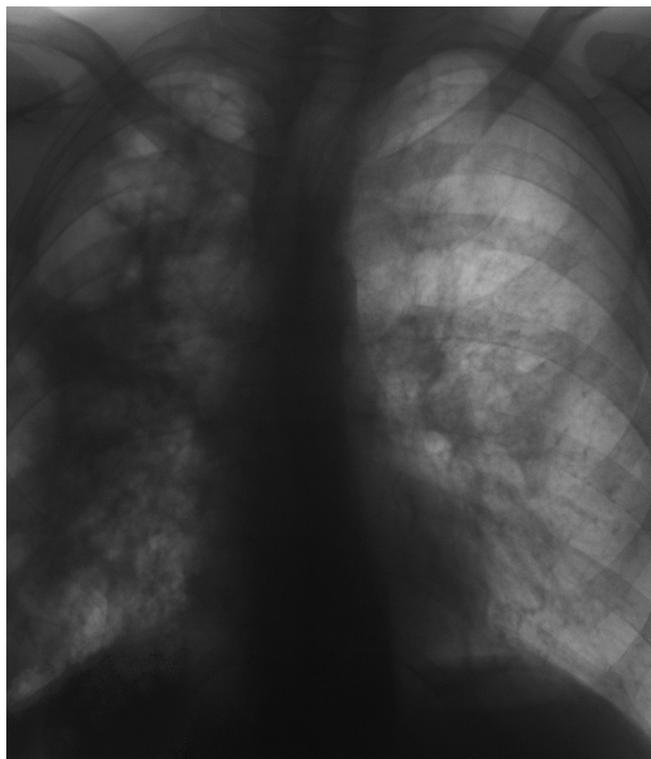


Fig. 1. Chest X-ray on hospital admission

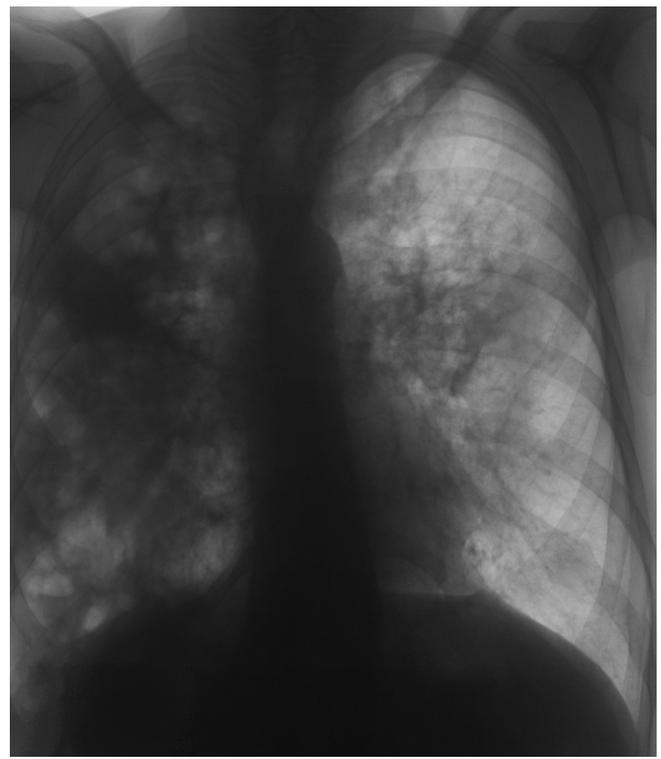


Fig. 2. Chest X-ray four months after starting TB treatment

With equal distributions of two samples according to Fisher's exact test, it can be seen that a statistically significant break in treatment is most often found in patients who had a repeated episode of treatment, which is confirmed by the reliability of the value $p=0.051$, more effective treatment according to the cured status was in patients with the first episode of treatment, shows

reliability value $p=0.05$. According to other criteria of treatment effectiveness, such as failure, prolongation of treatment duration, hearing loss, palliative treatment, the same frequency of manifestation was observed both in the first and in the second episodes of treatment with the reliability of the value of the Fisher test, respectively $p=0.431$, $p=0.432$, $p=0.69$, $p=0.13$. To correct

ototoxicity manifestation and prevent its progression, detoxification therapy, adenosine-triphosphoric acid (ATP), B vitamins, hepatoprotectors were administered. Vascular agents (cavinton, nootropil) were not used as they enhance the toxic effect of anti-TB drugs in the intensive phase of therapy. However, despite all the efforts made to control adverse effects, at the end of the first episode of MDR-TB treatment, 50% of convalescents reported hearing impairment, with the treatment success rate of 80.0%. No statistic reflects an individual case; hence we present a case report of pronounced toxicity when the patient refused TB treatment. A 35-year-old musician M. was admitted to inpatient treatment. The patient presented with coughing up white mucus, subfebrile temperature of 37.5 °C, general fatigue, weight loss, dyspnea on exertion. CBC: hemoglobin (Hbg) – 116 g/l, red blood cells (RBC) – $3.1 \times 10^{12}/l$, white blood cells (WBC) – $12.6 \times 10^9/l$; band neutrophils – 7%, segmented neutrophils – 65%, monocytes – 10%; lymphocytes – 12%; eosinophils – 6%; erythrocyte sedimentation rate (ESR) – 28 mm/hr. Chest X-ray confirmed disseminated destructive TB (Fig 1). Recurrent MDR-TB was diagnosed: resistance I (isoniazid, rifampicin, ethambutol), resistance II (-). The following treatment regimen was created: 8 Z Lfx Km Eto Cs PAS /12 Z Lfx Eto Cs PAS.

During the first month of treatment, dizziness and hearing loss were observed. According to audiometry findings (mild SHL) and after ENT consultation, B vitamins, specifically B₁, B₆, B₁₂, at a dose of 2.0 ml every other day, 1 ml of 1% ATP solution intramuscularly for 10 days, and Heptral (1 capsule twice a day) were added to the treatment regimen. The treatment received did not normalize hearing loss. A one-week interruption of TB treatment and detoxification were suggested. Upon returning to the treatment regimen, hearing loss worsened – moderate SHL according to audiometry findings. The patient refused Km injections. The efficacy of further TB therapy reduced and three weeks after the termination of Km injections, intoxication symptoms, including fever up to 38.6 °C, general fatigue, dyspnea on exertion developed. CBC: Hbg – 115 g/l, RBC – $3.1 \times 10^{12}/l$, WBC – $13.6 \times 10^9/l$; band neutrophils – 7%, segmented neutrophils – 67%, monocytes – 10%; lymphocytes – 10%; eosinophils – 6%; ESR – 34 mm/hr; controlled chest X-ray was negative (Fig 2).

A decision on returning to the treatment regimen with Km was made. The patient agreed to change therapy. However, within the first month of returning to the standard TB treatment regimen, hearing loss worsened again. Despite the improvement of the general condition, the patient signed refusal of treatment form, was transferred to the category of palliative care, and left for another region.

The discovery of new antimycobacterial agents (linezolid, delamanid, bedaquiline, pretomanid) allowed for shortening treatment duration, modifying the regimens, and improving treatment efficacy up to 90.0%. Aminoglycosides are used only in cases when there is a need for an individualized treatment plan. Such approach significantly reduced the incidence of ototoxicity. Thus, during 2020-2022, 181 reports on adverse effects were documented: 139 (76.8%) adverse events were reported by patients receiving MDR-TB treatment and only four (2.9%) patients developed ototoxicity (individualized therapy with Km).

In patients with manifestations of ototoxicity, prolongation of the duration of treatment was noted during repeated treatment in 2 (100.0%) patients, a clinically significant decrease in hearing was observed in 1 (50.0%) patient during a repeated treatment episode.

These cases were successfully corrected by replacing the preparation by another agent from Group C. The 11-month modified short-term treatment regimen including bedaquiline (for 6 months), levofloxacin, clofazimine, ethionamide, ethambutol, isoniazid (high dose), pyrazinamide + levofloxacin, clofazimine, ethambutol, and pyrazinamide for 5 months produced no ototoxic effects.

DISCUSSION

TB is a very 'stubborn' disease which remains one of the most dangerous infectious killers worldwide. An understanding of mutual responsibility of the society and the need to make every effort possible to control the disease encourage constant progress in the development and improvement of both diagnostic methods and treatment regimens.

The main challenge is effective therapy which results in treatment success without any side effects being barriers in TB treatment and leading to unfavorable outcomes. Some adverse effects develop slowly, and the patient seems to adapt to changes, believing that they are the consequences of TB and that they will go away with recovery. Unfortunately, the outcomes are unfavorable as having a disability does not mean a person has good health. In some cases, however, serious adverse event develops, which progresses and results in patient's refusal of TB treatment [7,13].

Authors Ignat'eva V.I., Martysyniuk T.M. (2018) pay special attention to the negative effects of polychemotherapy on the central and peripheral nervous system. At the same time, special importance is attached to drugs of the group of aminoglycosides, which cause damage to the vestibule-cochlear nerve. It is noted that during the main course of chemotherapy in patients with MDR

TB of the lungs, medication complications occur, which are manifested by the «hearing loss» syndrome, namely: acute sensorineural deafness, bilateral eustachianitis, and drug-induced encephalopathy. Accordingly, there is a need to use additional methods of differential diagnosis: echoencephalography and audiometry. This makes the course of tuberculosis treatment more expensive and not always available on time [7]. Complaints related to hearing loss, as a rule, appear already at the stage of deep and irreversible damage to the cochlear nerve. This leads to deterioration of treatment results. The data obtained by us are comparable to the data of the above-mentioned authors. Moreover, among patients treated between 2017 and 2019, break in treatment is most often found in patients who had a repeated episode of treatment, which is confirmed by the reliability of the value $p=0.051$, more effective treatment according to the cured status was in patients with the first episode of treatment, shows reliability value $p=0.05$. Modern antimycobacterial agents and new, modified, short-term treatment regimens eliminate a lot of side effects leading to a deterioration in the patients' condition, treatment termination, and transfer to the category of palliative care [1,8,10]. The analysis of data on the efficacy of treatment and the incidence of ototoxicity as a side effect during the observed periods found that during 2017-2019, adverse events were significantly more common as compared to 2020-2022 – 21.0% and 2.9%, respectively. Among the cohort of patients with manifestations of ototoxicity in the period 2020-2022, only a prolongation of the treatment period was observed, which did not lead to palliative treatment. Also, in only one patient from the group of repeated episodes of therapy hearing loss was observed.

Among patients receiving the individualized treatment regimen, who periodically underwent detoxification and pathogenetic therapy due to adverse effect development, the high percentage of cases with longer treatment duration was noted.

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CONCLUSIONS

1. Antimycobacterial therapy of multidrug-resistant pathogen strains with the addition of aminoglycosides increases the incidence of ototoxic adverse effects. This has the negative effect on the duration and efficacy of treatment.
2. A high frequency of ototoxic lesions was observed in patients with multidrug-resistant tuberculosis who were treated with aminoglycosides during the period 2017-2019 – 21,0%. The development and implementation of modified short-term treatment regimens for MDR-TB, in the period 2020-2022, minimizes the occurrence of further adverse events such as SHL - 2,9%, thereby allowing the patient to recover from TB without developing serious complications such as total hearing loss.
3. The development of ototoxic lesions led to the negative effect of treatment. Moreover, repeated cases of treatment were characterized by more significant changes. We noted that a statistically significant break in treatment is most often found in patients who had a repeated episode of treatment, which is confirmed by the reliability of the value $p=0.051$, more effective treatment was in patients with the first episode of treatment, shows reliability value $p=0.05$. Failure, prolongation of treatment duration, hearing loss, palliative treatment, was with the same frequency of manifestation was observed both in the first and in the second episodes of treatment with the reliability of the value of the Fisher test, respectively $p=0.431$, $p=0.432$, $p=0.69$, $p=0.13$.
4. TB patients are recommended to be adapted to short-term drug regimens as much as possible by assessing the risks of adverse effects and their preventing to avoid treatment termination as the next step is the creation of the individualized treatment plan which may include aminoglycosides, if there is no other choice. Therapeutic drug monitoring, timely detection of adverse effects, their prevention and treatment are the keys to cure the patient.

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MORPHOMETRIC CHARACTERISTICS OF PLACENTA IN WOMEN WITH PREECLAMPSIA AND OBESITY COMPARED TO WOMEN WITH NORMAL BODY WEIGHT

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ABSTRACT

The aim: to investigate the morphometric characteristics of placentas in women with comorbidity of preeclampsia and obesity compared to women with physiological body weight, and to assess the efficacy of the prophylactic therapy course developed to prevent the occurrence of preeclampsia in pregnant women with obesity.

Materials and methods: 25 biopsy samples of placental tissue were taken from women between 37 and 40 weeks of gestation with a physiological body weight and with class II obesity. The women were divided into five groups of five women in each: the 1st group included women with physiological body weight without obstetric and somatic pathology; the 2nd group involved women with physiological body weight, whose pregnancy was complicated with preeclampsia; the 3rd group was made up of women with class II obesity whose pregnancy was complicated with preeclampsia; the 4th group consisted of women with class II obesity, who received the special prophylactic therapy course, and the 5th group included women with class II obesity, who did not receive the prophylactic therapy course.

Results: The analysis of morphometric parameters of placenta samples taken from women with preeclampsia and obesity demonstrates a number of compensatory and adaptive changes in placenta under hypoxic conditions, and the most important of them include a significant decrease in the number and the mean diameter of the terminal villi, the reduction of volume of villous tree, an increase in the diameter of the capillaries of terminal villi. The morphometric parameters and histological structure in placenta samples from women with obesity, who received the special the prophylactic therapy course, as well as in placenta samples of the control group were similar to the gestational normative values.

Conclusions: The morphometric investigation of placenta samples taken from women with comorbidity of preeclampsia and obesity has shown a significant decrease in the mean diameter of the terminal villi and an increase in the diameter of the capillaries of these terminal villi when compared with a group of women with preeclampsia and physiological body weight. The study has also demonstrated the distortion of the percentage ratio of the volume of the intervillous space and the ratio of medium-calibre villi. The combination of these changes indicates a lack of adaptive capabilities in the placenta during preeclampsia under increasing hypoxic condition.

KEY WORDS: preeclampsia, pregnancy, placenta, obesity, prophylactic therapy course

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INTRODUCTION

The incidence of obesity and overweight among pregnant women is continuing to climb from year to year. The number of pregnant women with obesity in economically developed countries reaches 16-38% and is constantly increasing [1]. Obesity seriously impacts on the demographic situation contributing to infertility and morbidity rate since the complex of hormonal and metabolic disorders inherent in this condition often appears as the pathogenetic basis of the reproductive health deterioration in women and as a risk factor of both maternal and neonatal morbidity and mortality [2]. Among the complications compromised by obesity that lead to maternal mortality is preeclampsia (PE), a

common and multisystem disorder. However, despite decades of intensive research, data on the PE aetiology, and in particular, in comorbidities, are still limited. The common mechanisms underlying the development of obesity and PE comorbidity and its progression include oxidative stress, systemic inflammatory response syndrome, and placental circulation disorders [3]. These disorders cause morpho-functional disturbances in the components of the mother-placenta-foetus system. Chronic fetoplacental insufficiency is associated with various obstetric disorders and almost always leads not only to PE, but also to delayed foetal growth and premature birth [4]. Therefore, the investigation of adaptive and pathological changes in the structure

of the placenta may be particularly beneficial in explaining the causes underlying the PE development under concomitant obesity, as well as in evaluating the efficacy of agents with antioxidant, anti-inflammatory, and angioprotective properties that improve hemomicrocirculation and prevent the PE occurrence.

THE AIM

The aim of the study is to investigate the morphometric characteristics of placentas in women with comorbidity of preeclampsia and obesity compared to women having a physiological body weight, and to assess the efficacy of the prophylactic therapy course developed to prevent the occurrence of preeclampsia in pregnant women with obesity.

MATERIALS AND METHODS

For study, 25 biopsy samples of placental tissue were taken from women between 37 and 40 weeks of gestation with a physiological body weight (BMI – 18.5 – 24.9 kg/m²) and with class II obesity (BMI – 35 – 40 kg/m²), whose course of pregnancy was complicated with the PE development. The women were divided into five groups of five women in each: the 1st group included women with physiological body weight without obstetric and somatic pathology; the 2nd group involved women with physiological body weight, whose pregnancy was complicated with mild PE; the 3rd group was made up of women with class II obesity whose pregnancy was complicated with mild PE; the 4th group consisted of women with class II obesity, who received a prophylactic therapy course (PTC) we developed for the purpose to prevent PE; and the 5th group included women with class II obesity, who did not receive PTC. The main criteria for inclusion in the study were: spontaneous or induced pregnancy, singleton pregnancy, mild PE, timely delivery. Exclusion criteria were: severe PE, severe extragenital pathology, premature birth, multiple pregnancy, in vitro fertilization, antenatal and intranatal fetal death, manual separation of the placenta.

Since pregnant women with obesity are at risk of the PE occurrence, their pregnancy was under special medical supervision in accordance to the clinical guideline for obstetric care “Hypertensive disorders during pregnancy, childbirth and the postpartum period” (Order of the Ministry of Health of Ukraine No. 151, 01.24.2022). In addition to the recommendations regulated by this order, pregnant women with obesity received the PTC, which we specifically developed. It included a combination of L-arginine and semi-synthetic diosmin taken according to the following scheme over

12 – 16, 22 – 26, and 32 – 36 weeks of gestation. Per oral dose of diosmin, which is produced in the form of tablets, was 600 mg per day. L-arginine produced as syrup was taken in a dose of 5 ml three times a day.

For morphometric study, biopsy samples sized 2.0×1.5×1.0 cm were excised from the central, paracentral, and marginal areas of the maternal part of the placenta and then were fixed in a 10% neutral formalin solution. Following the fixation and dehydration with increasing concentrations of ethanol, biopsies were embedded in paraffin blocks. Histological sections cut by a standard microtome at 5 µm thickness were stained with hematoxylin and eosin. The sections were analyzed using an *Olympus BX 41 light microscope* (Japan) with a digital camera and a set of licensed programs. To obtain stereometric data, we applied the planimetric method and the point count by placing a grid with a 3 µm graticular division at magnification 400×. We carried out morphometric study to evaluate the following parameters in the terminal chorionic villi sized up to 80 µm: the diameter of the terminal villi; diameter of capillaries of terminal villi; the thickness of the syncytiotrophoblast (STB); the volume fraction of the main components of the placenta including stem villi, medium-calibre villi, terminal villi, and intervillous space.

Conducted prospective clinical and statistical analysis of pregnancy and childbirth histories (form No. 096/o) and medical records of newborns (form No. 097/o) approved by the Order of the Ministry of Health of Ukraine «On Amendments to Primary Registration Forms and Instructions for Their Filling», No. 29, 01.21.2016.

The statistical analysis of the findings obtained was performed by the “MedStat” program using the methods of descriptive statistics, calculating the mean sample values (M) and the error of the mean value (m), qualitative indicators given as frequencies and their percentage ratios. The Mann-Whitney U-test was used to assess intergroup differences. Differences at $p < 0.05$ were considered as statistically significant for analysis.

RESULTS

The morphometric analysis of histological preparations of placental samples taken from the women with physiological body weight and with class II obesity whose pregnancy was complicated by the mild PE development reveals the deviation in the parameters of the test groups from the respective parameters of the control group (Table I).

The mean diameter of the terminal placental villi in the women with PE and class II obesity was significantly lower compared with the indicators in the group of women with PE and physiological body weight (32.66 ± 1.22 µm

Table I. Comparative characteristics of the main morphometric parameters of the terminal placental villi in the studied groups

Morphometric parameters, μm	Women with physiological body weight, n=5	Women with PE and physiological body weight, n=5	Women with PE and class II obesity, n=5	Women with class II obesity, who received PTC, n=5	Women with class II obesity, who did not received PTC, n=5
Mean d of terminal villi	53,75 \pm 2,39	36,52 \pm 1,16 p_1	32,66 \pm 1,22 p_2, p_3, p_4	53,66 \pm 0,11	48,44 \pm 0,37 p_6
Meas d of capillaries	11,94 \pm 0,35	14,44 \pm 0,21 p_1	15,43 \pm 0,21 p_2, p_3, p_4	12,42 \pm 0,15 p_5	13,9 \pm 0,04 p_6
STB thickness	5,96 \pm 0,76	5,53 \pm 0,41	5,06 \pm 0,25 p_2, p_3	6,03 \pm 0,58	6,37 \pm 1,05 p_6

Note:

p_1 – reliability when comparing the indicators between the 1st and 2nd groups, $p < 0.05$;

p_2 – reliability when comparing the indicators between the 1st and 3rd groups, $p < 0.05$;

p_3 – reliability when comparing the indicators between the 5th and 3rd groups, $p < 0.05$;

p_4 – reliability when comparing the indicators between the 2nd and 3rd groups, $p < 0.05$;

p_5 – reliability when comparing the indicators between the 1st and 4th groups, $p < 0.05$;

p_6 – reliability when comparing the indicators between the 4th and 5th groups, $p < 0.05$.

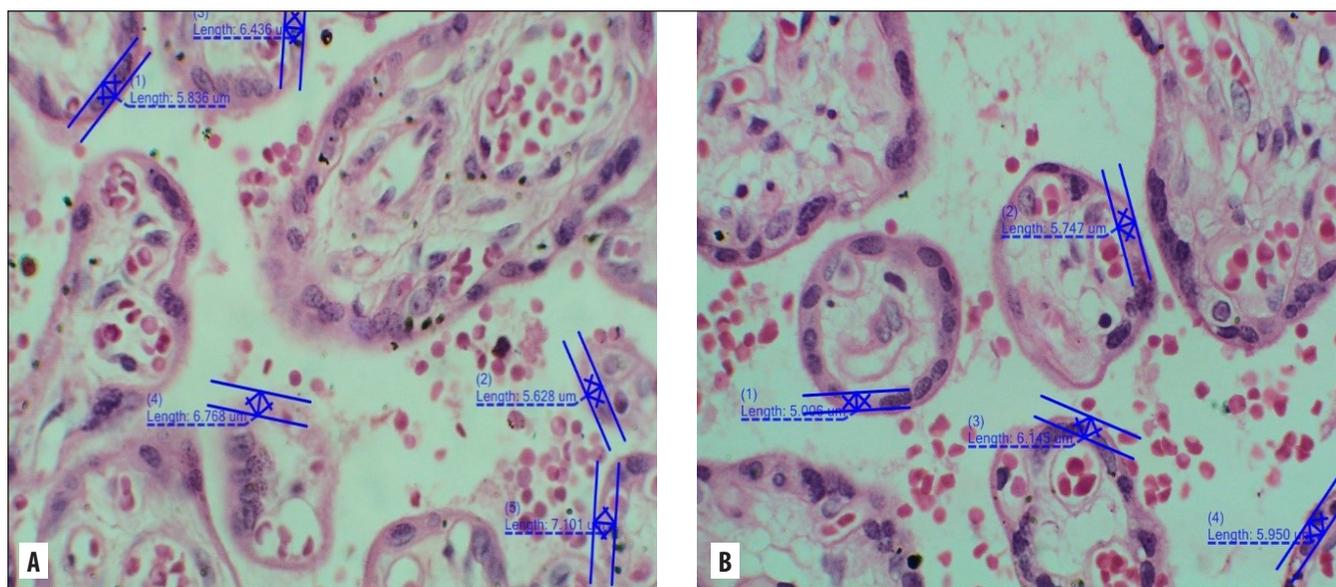


Fig. 1. Placenta biopsy samples taken from women with class II obesity A) who did not receive PTC; B) taken from women who received PTC. The thickness of the syncytiotrophoblast of the terminal villi. Hematoxylin and eosin staining; magnification: x400.

vs. 36.52 \pm 1.16 μm , respectively, $p_4 < 0.01$). A significant decrease of this indicator was observed in the placental samples taken from women with PE and physiological body weight compared with the group of placental biopsies taken from women with physiological body weight and normal course of pregnancy (36.52 \pm 1.16 μm vs. 53.75 \pm 2.39 μm , $p_1 < 0.01$). The same tendency was observed when comparing the mean diameter of terminal villi between the placentas of women with PE and class II obesity and the group of women with class II obesity, whose pregnancy was not complicated by the PE course (32.66 \pm 1.22 μm vs. 48.44 \pm 0.37 μm , $p_3 < 0.01$). There was a significant decline in the mean diameter of the terminal villi of placentas from women with concomitant class II obesity when compared with the indicators of

the control group (48.44 \pm 0.37 μm vs. 53.75 \pm 2.39 μm , $p_6 < 0.01$) (Fig. 1). Which indirectly testifies to the connection of a number of pathological changes and deviations of morphometric parameters in placentas from women with moderate preeclampsia and women with obesity, which in turn proves the fact that preeclampsia is manifested by obesity and the feasibility and advantages of prescribing PTC to pregnant women with accompanying obesity for the purpose of prevention of PE.

At the same time, the capillaries of terminal villi in placenta samples taken from women with PE and class II obesity had a larger diameter than in the group of women with PE and physiological body weight (15.43 \pm 0.21 μm vs. 14.44 \pm 0.21 μm , $p_4 < 0.01$). An increase in the diameter of the capillaries of terminal villi was also found

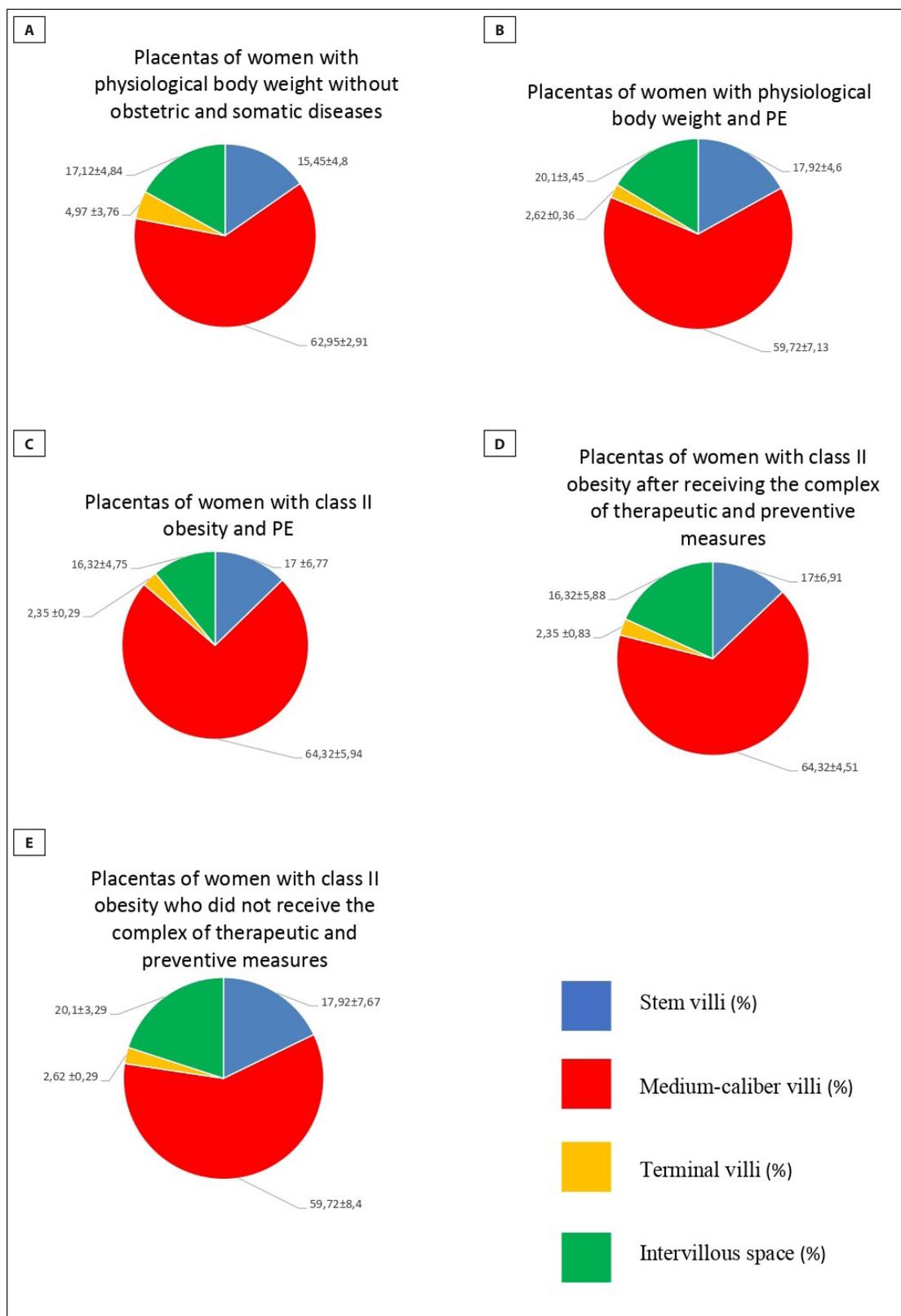


Fig. 2. Comparative characteristics of the relative volume of the main placental components in the studied groups A) Placentas of women with physiological body weight without obstetric and somatic diseases; B) Placentas of women with physiological body weight and PE ; C) Placentas of women with class II obesity and PE; D) Placentas of women with class II obesity after receiving the complex of therapeutic and preventive measures; E) Placentas of women with class II obesity who did not receive the complex of therapeutic and preventive measures.

in placenta samples from women with PE and physiological body weight, compared to the placenta samples from women of the control group ($14.44 \pm 0.21 \mu\text{m}$ vs. $11.94 \pm 0.35 \mu\text{m}$, $p_1 < 0.01$). There was a similar pattern found when comparing the indicators between the 3rd group and the 5th group ($15.43 \pm 0.21 \mu\text{m}$ vs. 13.9 ± 0.04

μm , $p_3 < 0.01$). An increase in the diameter of the capillaries of the placental terminal villi is seen in women with class II obesity compared to the control group ($13.9 \pm 0.04 \mu\text{m}$ vs. $11.94 \pm 0.35 \mu\text{m}$, $p_6 < 0.01$) (Table I).

The mean thickness of STB was slightly greater in the placenta samples from women with PE and physiologi-

cal body weight compared with the indicators in the group of women with PE and class II obesity ($5.53 \pm 0.41 \mu\text{m}$ vs. $5.06 \pm 0.25 \mu\text{m}$, respectively, $p > 0.05$). When comparing the indicators of the mean thickness of STB between the group of women with PE and physiological body weight and the indicators of the control group, we observed a tendency to their decline, but no significant difference was determined ($5.53 \pm 0.41 \mu\text{m}$ vs. $5.96 \pm 0.76 \mu\text{m}$, respectively, $p > 0.05$). A significant decrease of this indicator was revealed in the group of women with PE and class II obesity compared to the indicators in the group of women with class II obesity ($5.06 \pm 0.25 \mu\text{m}$ vs. $6.37 \pm 1.0 \mu\text{m}$, respectively, $p_3 < 0.01$). A smaller thickness of STB was found in the placentas of women in the control group than in the placentas of women with II degree obesity ($5.96 \pm 0.76 \mu\text{m}$ vs. $6.37 \pm 1.0 \mu\text{m}$, respectively, $p_6 < 0.02$).

The morphological study of the volume fraction of the main placenta components demonstrates that the volume of stem villi in placentas makes up $12.42 \pm 6.67\%$ in women with comorbidity of PE and class II obesity, $17.0 \pm 4.6\%$ in women with PE and physiological body weight; this indicator in the reaches $17.92 \pm 7.67\%$ in group of women with class II obesity and $15.45 \pm 4.8\%$ in the control group. The percentage of medium-calibre villi in the placentas of women with comorbidity of PE and class II obesity is $71.32 \pm 5.94\%$, and $59.72 \pm 7.13\%$ in women with physiological body weight, whose pregnancy was complicated by the PE. The percentage of medium-calibre villi in the placentas of women with class II obesity is $59.72 \pm 8.4\%$, and in women with a physiological body weight and a normal course of pregnancy is $62.95 \pm 2.91\%$. The volume of placental terminal villi makes up $2.62 \pm 0.29\%$ in women with the comorbidity of PE and obesity, $2.35 \pm 0.36\%$ in women with PE and physiological body weight, $2.62 \pm 0.29\%$ in women with class II obesity; this indicator in the control group is $4.97 \pm 3.76\%$. The indicators of the relative volume of the intervillous space are significantly lower in the placentas in the groups of women with PE and concomitant obesity and in women with PE and physiological body weight ($16.32 \pm 4.75\%$ and $20.1 \pm 3.45\%$, respectively, $p < 0.05$); in the placenta samples of women with class II obesity, this indicator is at the level of $20.1 \pm 3.29\%$, and in the placentas of the control group it equals to $17.12 \pm 4.84\%$ (Fig. 2).

Having analyzed the findings of the morphometric study of placenta samples from women with class II obesity, who received PTC, we can suggest that they are comparable to the control group and approaching the normal gestational parameters (Fig. 2).

The mean diameter of the terminal placental villi from women with class II obesity, who received PTC is

$53.66 \pm 0.11 \mu\text{m}$ that is higher than in the placentas of women who did not receive PTC ($53.66 \pm 0.11 \mu\text{m}$ vs. $48,44 \pm 0.37 \mu\text{m}$, respectively $p < 0.01$). The diameter of the placental capillaries in women who took the course of PE prevention is $12.42 \pm 0.15 \mu\text{m}$ that is similar to the respective indicators in the control group. The mean thickness of STB is smaller in women who received PTC compared to that in women who did not receive PTC during the pregnancy ($6.03 \pm 0.58 \mu\text{m}$ vs. $6.37 \pm 1.05 \mu\text{m}$, respectively $p > 0.05$) (Table I). The morphometric study of the volume fraction of the main components of the placenta demonstrated that the volume of stem placental villi in women with class II obesity, who received PTC, makes up $12.92 \pm 6.91\%$. The percentage of medium-calibre villi in the placentas of women who took PE prevention is $66.1 \pm 4.51\%$, and the volume of terminal villi in this group is $2.8 \pm 0.83\%$. The indicator of the volume fraction of the intervillous space in the placentas of women who received PTC equals to $18.27 \pm 5.88\%$ (Fig. 2).

Thus, the terminal placental villi in women with class II obesity and normal body weight, whose pregnancy was complicated by the mild PE development differ from those in women with physiological body weight and a normal course of pregnancy by a number of morphometric parameters including the diameter of the villi and their capillaries, STB thickness, changes in the parameters of the volume fraction of the main components of the placenta. It is worth to point out the morphometric placenta indicators of women with class II obesity, who received PTC during pregnancy, approach to the normal gestational parameters and can be comparable with the indicators of women with a physiological body weight and a normal course of pregnancy. The placentas from women with obesity who took PTC during the pregnancy have been found to show minor deviations of morphometric indicators and moderately expressed compensatory and adaptive reactions that correspond to normal gestational indicators. This proves the efficacy and feasibility of prescribing PTC to pregnant women with obesity in order to prevent the PE development.

DISCUSSION

In mild PE, premature placental aging accompanied with pronounced involutive-dystrophic processes can develop as opposed to the placental development during normal pregnancy that leads to fibrinoid necrosis and sclerosis of terminal villi with the subsequent occurrence of local ischemic infarctions of the placenta tissue [5]. Disorders of the normal histological structure of the placenta resulted from defective (delayed or early

maturation) maturation of chorionic villi are especially pronounced when PE develops in women with obesity.

A number of morphological changes detected in the placenta samples taken from women with mild PE demonstrate the impaired villi development and growth with an increasing angiogenic pattern, a decrease in the size of terminal villi, a reduced volume of the villous tree, which includes stem, intermediate and terminal villi. These changes are caused by the development of uteroplacental hypoxia and, as a consequence, malperfusion of the intervillous space [6]. Similar changes most likely cause lower indicators of placenta mass in pregnant women with obesity [7]. Since the main cause for the reduction in uteroplacental blood circulation in early-onset PE is considered to be the slowing down of the second wave of trophoblast invasion following 16 weeks of gestation, it is the incomplete invasion of cytotrophoblast into the uterine tissue that leads to incomplete transformation of the spiral arteries and a decreased inflow of maternal blood to the placenta with the further development of uteroplacental hypoxia [8]. Under pre-placental and utero-placental hypoxia, the extensive angiogenesis with vascular branching needed to increase the area for gas exchange of the villous tree is seen. The activation of pro-angiogenic factors is considered the main mechanism that triggers compensation processes in the villous tree of the placenta during hypoxia. Vascular endothelial growth factor (VEGF), placental growth factor (PIGF) and their receptors (VEGFR-1 and VEGFR-2) play the exceptionally important role in the formation of fetoplacental angiogenesis. Their balanced interaction determines the processes of vascularisation in villi in the early stages of pregnancy and the final formation of their capillary network, therefore, the development of either early-onset or late-onset PE is accompanied by a different degree of expression of angiogenic growth factors [9, 10]. The use of PTC we developed enables us to act on the term of PE manifestation that can be explained by both the PTC constituent components and the scheme of its medication regimen. According to our recommendations, the 1st PTC course lasts from the 12th to the 16th week of gestation as the process of interstitial cytotrophoblast invasion begins from the 16th to the 18th week of gestation. Impairment of this process is subsequently accompanied by the destruction of the elastomuscular components of vessels and their replacement with fibrinoid that results in deterioration of contractility of the placental vessels [11]. Therefore, the use of semi-synthetic diosmin, which has capillary protective, anti-edematous, anti-fibrinolytic, and anti-inflammatory effects, can prevent these adverse processes. And given the fact that the devel-

opment of early-onset and late-onset PE is manifested with different expression of angiogenic growth factors, the use L-arginine, which is able to reduce the concentration of antiangiogenic growth factors and promote angiogenesis, which is essential for increasing the area of gas exchange of the villous tree, seems to be quite appropriate [12-15]. Hence, the balanced interaction of the medicines promotes the processes of vascularisation of the villi in the early stages of pregnancy and the final formation of their capillary network and thus helps to prevent early-onset PE.

The following alterations including a decrease in the diameter of the terminal villi and an increase in the diameter of their capillaries, an increase in the STB thickness, a reduction of the volume fraction of the stem and terminal villi, a growth in the percentage ratio of medium-calibre villi and a decrease in the volume fraction of the intervillous space indicate a deteriorating placental circulation and a decrease in the intervillous perfusion that initiates the development of adaptation and compensation processes. A significant deviation of the morphometric parameters of the terminal villi from the control level suggests the fact of insufficiency of compensatory and adaptive reactions in the placenta during mild PE under increasing hypoxic conditions. Hypovascularisation (reduction in the number of vessels and their mean diameter), the STB thinning, increase in syncytiocapillary membranes, impairment of the differentiation of the vascular-stromal component in the villous chorion and increased fibrin deposition in the epithelium of these villi along with hypofunction of the terminal villi have also been revealed by other scientists in cases of delayed fetal growth, and in cases complicated with PE that evidences the failure of compensatory changes at the stage of chronic placental insufficiency [16]. Thinning of the cellular trophoblast of the chorion we found in the groups of women with mild PE is in good agreement with the reports of other scientists and can be found in other pathological conditions, besides anaemia [17]. The positive changes we obtained after applying PTC in the percentage ratio of the volume fraction of the main components of the placenta support previous findings in the literature that point out a significant growth in the area of capillaries of terminal villi and syncytiocapillary membranes in the placentas of women who received L-arginine aspartate [18].

The analysis of the morphometric parameters of the small-calibre arteries of the placenta in anaemia demonstrates an increase in the outer diameter of these vessels that evidenced a pronounced decrease in the permeability of small-calibre vessels, which are under greater functional stress compared to larger arteries and are the first and

more pronounced to be affected in various pathological conditions [19]. These authors confirm the damage to a significant number of endotheliocytes that leads to their dysfunction, a decrease in the synthesis of nitric oxide (NO), blockade of NO synthase, activation of NO degradation processes that is accompanied by spasm and narrowing of blood vessels. The latter worsens blood supply to organs, maintains and intensifies hypoxia, which is complicated by oedema, dystrophy, necrobiosis of tissues and cells (under significant damage to endotheliocytes, the degree of morphological changes in the placenta is more pronounced) that yet again proves the need to use a combination of an NO donor and an angioprotector, which are the constituent agents of PTC we propose.

CONCLUSIONS

The morphometric investigation of placenta samples taken from women with comorbidity of mild preeclampsia and obesity has shown a significant decrease in the mean diameter of the terminal villi ($32,66 \pm 1,22 \mu\text{m}$ vs. $36,52 \pm 1,16 \mu\text{m}$, $p < 0,01$) and an increase in the diameter of the capillaries of these villi ($15,43 \pm 0,21 \mu\text{m}$ vs. $14,44 \pm 0,21 \mu\text{m}$, $p < 0,01$) when compared with a group of women with preeclampsia and

physiological body weight. The study has also demonstrated the distortion of the percentage ratio of the volume of the intervillous space ($16,32 \pm 4,75\%$ and $20,1 \pm 3,45\%$, respectively) and the ratio of medium-calibre villi ($64,32 \pm 5,94\%$ and $59,72 \pm 7,13\%$, respectively). The combination of these changes indicates a lack of adaptive capabilities in the placenta during preeclampsia under increasing hypoxic condition.

The prophylactic therapy course, which combines L-arginine aspartate and semi-synthetic diosmin to reduce the occurrence of preeclampsia and other complications during pregnancy than may develop as a consequences of placental dysfunction under obesity, has been proven to reduce the structural, functional, and dystrophic changes of the placenta and bring the respective indicators in proximity to the normal values.

PROSPECTS FOR FURTHER INVESTIGATION

The easy of use, accessibility, and convincing positive results of the described method of pharmacological correction enables us to recommend this PTC for enhancing the effectiveness of the PE prevention in pregnant women with obesity thus preventing the incidence of obstetric and prenatal complications.

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ONE-STAGE PROSTATECTOMY ACCOMPANIED BY HERNIOPLASTY TO IMPROVE QUALITY-OF-LIFE OUTCOMES OF PATIENTS WITH COMBINED SURGICAL PATHOLOGIES

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ABSTRACT

The aim: To evaluate whether simultaneous inguinal hernioplasty during prostatectomy confers benefits on quality-of-life outcomes.

Materials and methods: 152 patients with prostatic adenoma were observed. The general group included 32 (21%) patients with prostatic adenoma and hernia inguinalis, who underwent one-stage prostatectomy accompanied with hernioplasty. 120 (79%) persons of comparison group underwent a prostatectomy only. The average age of the comparison group was 68.0 ± 7.0 years, the general group - 67.1 ± 6.9 years; the average prostate volume was 94.4 ± 42.3 cm³ and 91.2 ± 32.6 cm³ respectively. Hernia inguinalis was in 32 patients of the general group, in 4 of them - on both sides (36 cases totally).

Results: The average time of retropubic prostatectomy in both groups was the same, and simultaneous hernioplasty took 35.0 ± 17.4 minutes. The frequency of early and late bleeding after prostatectomies in the general group was 6.25% (2 cases) and 7.5% (9 cases) in the comparison group. No deaths were noted in two groups. The quality-of-life outcomes after the one-stage prostatectomy accompanied with hernioplasty in 6 and 12 months were statistically better than before these operations.

Conclusions: Performing one-stage prostatectomy accompanied with hernioplasty does not worsen the immediate and long-term results of operation, instead it helps to eliminate two diseases at the same time from one surgical approach.

KEY WORDS: benign prostatic hyperplasia, inguinal hernia, prostatectomy, hernia repair, prostatectomy complications

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INTRODUCTION

The possibility of simultaneous inguinal hernioplasty during prostatectomy for prostatic adenoma and hernia inguinalis is a topical issue in both urology and abdominal surgery [1 - 3]. In the domestic literature, there are no data on performing simultaneous hernioplasty at the same time as prostatectomy, as well as the results of such type operations. It must be clear that simultaneous hernioplasty is better performed during one-time retropubic prostatectomy (rather than transvesical), when the bladder is not dissected and drained with suprapubic drainage, which significantly reduces the risk of retropubic and retroinguinal spaces infection. Unfortunately, open prostatectomies with retropubic access in Ukraine are performed in single clinics, and therefore there are no results of simultaneous inguinal hernioplasty.

THE AIM

The aim of the research is to evaluate whether simultaneous inguinal hernioplasty during prostatectomy

for prostatic adenoma and hernia inguinalis confers benefits on quality-of-life outcomes.

MATERIALS AND METHODS

This study was conducted at Vinnytsya National Pirogov Memorial Medical University, Vinnytsya, Ukraine, from January 2016 to January 2021. The research included 152 patients with prostatic adenoma who were operated on in the urological department of the Vinnytsya Regional Clinical Hospital named after M. I. Pirogov

Before entering the patient in the study protocol, a written voluntary agreement to participate in this study was obtained in accordance with the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects. The study protocol was approved by the Local Ethics Committee (LEC) of the Vinnytsya National Pirogov Memorial Medical University, protocol №8 dated 10/17/2019. All patients provided written informed consent. The general group included 32 (21%) patients who suffered from prostatic adenoma

and hernia inguinalis, who underwent one-moment retropubic prostatectomy accompanied with simultaneous pre-peritoneal inguinal hernioplasty. The number of members in the comparison group was 120 (79%) persons with prostatic adenoma who underwent a single retropubic prostatectomy only. The average age of the comparison group patients was 68.0 ± 7.0 years, the general group - 67.1 ± 6.9 years; the average prostate volume was 94.4 ± 42.3 cm³ and 91.2 ± 32.6 cm³ respectively. Urinary retention (acute or chronic) was the reason of urgent admission to the hospital in 44 (36.7%) patients of the comparison group and 4 (12.5%) of the general group. Hernia inguinalis was diagnosed in 32 patients of the general group, in 4 of them - on both sides (36 inguinal hernias totally). The distribution of patients in the general group by type and degree of inguinal hernia according to the EHS classification (2009) [4] is presented in Table I.

The obtained quantitative data were calculated by the variational statistics method using standard Microsoft Excel software packages on a personal computer with the calculation of the arithmetic mean M , the mean error of the mean m and the root mean square deviation σ , the Student's t-test and the difference between the data were considered reliable at a significance level of $p < 0.05$.

RESULTS

The average time of retropubic prostatectomy and simultaneous hernioplasty in general and comparison group is presented in Table II. The average time of retropubic prostatectomy in general and comparison groups was the same, and simultaneous hernioplasty took on an average 35.0 ± 17.4 minutes.

Intraoperative acute bleeding was observed in two patients (1.7%) from 120 of the comparison group, who lost 1.2 and 1.5 liters of blood. There was no intraoperative bleeding from the prostate bed in the general group. Intraoperative blood loss was determined by the gravimetric method [5, 6]. There were no patients in the general or comparison group who required reoperation due to early or late bleeding. The frequency of early and late bleeding after retropubic prostatectomies, which required blood transfusion, in the general group was 6.25% (2 cases) and 7.5% (9 cases) in the comparison group.

Postoperative complications were divided into early and late [5, 7, 8]. Early (immediate) postoperative complications mean complications after prostatectomy during inpatient treatment, late complications after discharge from the hospital (ambulatory period of supervision). The nature of early urological complications after one-stage retropubic prostatectomies in

two groups is presented in Table III. The neurological complications are illustrated in Table IV.

The most frequent complications after prostatectomy, including epididymitis (funiculitis), wound suppuration, chronic pyelonephritis exacerbation, pubic bones osteitis were not observed in both groups. No deaths were noted after the operations in two groups.

Classification of urological complications according to Clavien-Dindo after retropubic prostatectomy in patients of the general and comparison groups is presented in Table V.

As can be seen from Table V, postoperative complications occurred in every third patient of both groups. The majority of complications were presented in the I degree of Clavien-Dindo's classification. The average postoperative bed-day in patients of general group was 9.5 ± 1.7 ; in comparison group - 9.8 ± 2.9 .

DISCUSSION

To unify and systematize postoperative complications, surgeons R.A. Clavien et al. in 1992 proposed a new classification which was improved by D. Dindo et al. in 2004. It became world-famous and also began to be widely used after urological operations [5]. The Clavien-Dindo's classification takes into account not only the quantitative assessment of postoperative complications, but also the qualitative ones depending on the degree of complication severity. It can offer the type and volume of medical measures aimed at eliminating these complications. The Clavien-Dindo's classification determines the quantitative and qualitative assessment of postoperative complications within 90 days after surgery. Because it was recommended for use by the European Association of Urologists (EAU) and urological associations of other countries. Classification of postoperative complications according to Clavien-Dindo allows a better (more detailed) characterization of each complication and the volume of medical or repeated surgical care (under local or general anesthesia); compare and statistically evaluate urological and neurological complications of different urological clinics; more precisely determine the sequence of multimodal treatment; preliminarily change the provision of assistance to avoid or reduce complications; establish criteria for the assistance quality.

Thus, the average duration of retropubic prostatectomy in patients of the both groups was the same, like the postoperative bed-day. Despite the longer duration of the one-stage prostatectomy accompanied with hernioplasty, the frequency of postoperative complications was comparable in general and comparison groups. Long-term results of the one-stage

Table I. Distribution of patients in the general group by type and degree of inguinal hernia according to the EHS (2009) classification

Type of inguinal hernia	Degree of inguinal hernia			Number of patients	
	1	2	3	Absolute	%
Hernias of the lateral inguinal fossa (oblique or L) n=22	1 (L1)	14 (L2)	7 (L3)	19	59,4
Hernias of the medial inguinal fossa (direct or M) n=14	1 (M1)	7 (M2)	6 (M3)	13	40,6
Total: 36	2	21	13	32	100

Table II. The average duration of retropubic prostatectomy and simultaneous hernioplasty in the general group of patients and in the comparison group

Type of surgical intervention	Average duration of surgical intervention	
	General group (minutes)	Comparison group (minutes)
One-moment retropubic prostatectomy	82,8±25,6	80,1±17,4
Simultaneous hernioplasty	35,0±17,4	-

Table III. Early urological complications after retropubic prostatectomy in the general group of patients and in the comparison group

Early urological complications	Patients after retropubic prostatectomy	
	General group (n=32)	Comparison group (n=120)
Bleeding - early	2 (6,25%)	8 (6,7%)
- late	-	1 (0,8%)
- early and late together	2 (6,25%)	9 (7,5%)
Excretion of urine through a suprapubic wound	-	1 (0,8%)
Hyperthermia after removal of urethral drains	1 (3,1%)	7 (5,8%)
Stress incontinence	1 (3,1%)	2 (1,7%)
Acute urine retention	-	1 (0,8%)
Long-lasting (up to 10 days) serous leakage from the retropubic space	1 (3,1%)	-
Paravesical hematoma	-	1 (0,8%)
Wicket swelling	1 (3,1%)	-
Total	6 (18,75%)	21 (17,5%)

Table IV. Early neurological complications after retropubic prostatectomy in the general group of patients and in the comparison group

Early neurological complications	Patients after retropubic prostatectomy	
	General group (n=32)	Comparison group (n=120)
Thromboembolism of minor branches of the pulmonary artery	-	2 (1,7%)
Hypertensive crisis	1 (3,1%)	2 (1,7%)
Acute coronary syndrome	-	1 (0,8%)
Acute psychosis	-	1 (0,8%)
Stomach dyskinesia with pain syndrome	-	2 (1,7%)
Pneumonia	1 (3,1%)	-
Gouty arthritis exacerbation of the knee joint	-	1 (0,8%)
Total	2 (6,25%)	9 (7,5%)

prostatectomy accompanied with hernioplasty were studied by repeated outpatient examination in 1, 3, 6, and 12 months after surgery. Bladder stones, bladder neck and urethra strictures were not discovered in the

patients. One patient with stress urinary incontinence was treated conservatively (Nexetin 40 mg twice a day in combination with Kegel's exercises to strengthen the pelvic floor muscles) for 2 months, after which he

Table V. Classification of urological complications after retropubic prostatectomy according to Clavien-Dindo and their treatment in patients of general and comparison groups

Degree of complication	Complications (number of patients in absolute values and percentages) in the comparison group (n=120)	Complications (number of patients in absolute values and percentages) in the general group (n=32)	Treatment of complications
I	Hyperthermia after removal of urethral drainage (7 - 5.8%)	Hyperthermia after removal of urethral drainage (1 - 3.1%)	Foley Catheter Reinsertion, Antibacterial, Antipyretic, Anti-Inflammatory Therapy
	Urine excretion through the suprapubic wound (1 - 0.8%)	-	Repeated installation of the Foley catheter, antibacterial therapy
	Acute urinary retention (1 - 0.8%)	-	Repeated installation of the Foley catheter, antibacterial therapy
	-	Prolonged (up to 10 days) serous leakage from the retropubic space - 3.1%)	Dressings using local antiseptic solutions
	Paravesical hematoma (1 - 0.8%)	-	Dressings using local antiseptic solutions
	-	Wicket swelling (1 - 3.1%)	Anti-edematous, anti-inflammatory, antibacterial and pain-relieving therapy
	Stress urinary incontinence «d» (2 - 1.7%)	Stress urinary incontinence «d» (1 - 3.1%)	Kegel exercises, duloxetine
	Hypertensive crisis (2 - 1.7%)	Hypertensive crisis (1 - 3.1%)	Hypotensive therapy
	Gastric dyskinesia with pain syndrome (2 - 1.7%)	-	Fibrogastroduodenoscopy, spasmolytic, with painless therapy
	-	Pneumonia (1 - 3.1%)	Antibacterial, anti-inflammatory, antipyretic, detoxification, broncholytic therapy
Acute psychosis (1-1.71%)	-	Antipsychotic drugs	
Exacerbation of gouty arthritis of the knee joint (1-1.7%)	-	Anti-inflammatory, antipyretic, antibacterial therapy	
II	Early and late bleeding from the prostate bed (9 - 7.5%)	Early and late bleeding from the prostate bed (2 - 6.25%)	Hemostatic therapy, blood transfusion
	Postoperative anemia (4 - 3.3%)	Postoperative anemia (1 - 3.1%)	Blood transfusion
IIIa	-	-	-
IIIb	-	-	-
IVa	Postoperative hypotension (6 - 5%)	Postoperative hypotension (2 - 6.25%)	Treatment in the intensive care unit
	Acute coronary syndrome (1 - 0.8%)	-	Treatment in the intensive care unit
	Thromboembolism of minor branches of the pulmonary artery (2 - 1.7%)	-	Treatment in the intensive care unit
IVb	-	-	-
V	-	-	-
Total	40 (33,3%)	11 (34,2%)	

achieved complete urinary continence. In 3, 6, and 12 months after the operation, he completely retained urine during physical exertion (lifting heavy objects), coughing, sneezing, and brisk walking.

Long-term results of simultaneous hernioplasty and patients' quality of life were evaluated using the questionnaire of the European Society of Herniologists (EHS) - EuraHS -

QoL (European Registry of Abdominal Wall Hernias) [8]. To evaluate the long-term results of simultaneous pre-peritoneal inguinal hernioplasty, the EuraHS - QoL quality of life questionnaire is the most convenient. The SF - 36 Health Survey and its short form, the SF - 12, are not specific to concurrent operations as they assess general physical functioning, role functioning, body pain, general health, vitality,

Table VI. Evaluation of the quality of life after simultaneous pre-peritoneal hernioplasty of inguinal hernias

Type of urinary incontinence	Symptoms before surgery	After 6 months after the operation	Condition 12 months after surgery (urodynamic monitoring)
The total score of the EuraHS questionnaire - Quality of Life scale	64,8±5,7	34,5±5,1*	33,1±4,4*

Note. * - $p < 0.05$ compared with the preoperative indicator

social functioning, emotional state, and mental health. And such a severe surgical intervention as a prostatectomy can affect all domains of questionnaires. The next CCS (Carolinas Comfort Scale), which is popular among herniologists, assesses the impact of a mesh implant (sensation of the mesh, pain, restriction of various movements) on the patient's quality of life after surgery [9]. The evaluation of the pain symptom after simultaneous hernioplasty using the ASS questionnaire (ASS-visual analog school) is also not specific, since the patient underwent prostatectomy and the pain syndrome may be caused by urological surgery. The EuraHS - QoL questionnaire allows patient to assess the pain syndrome no earlier than 3 months after surgery [8].

In patients after retropubic prostatectomy, reparative processes in the area of operation and possible postoperative complications (strictures of the bladder neck or urethra, bladder stones) occur precisely in the period up to 3-6 months [5], and therefore, in the absence of complications after retropubic prostatectomy, pain syndrome after it is advisable to evaluate the placement of the mesh implant no earlier than 6 months after the simultaneous operation. We conducted a survey of patients after simultaneous hernioplasty using the EuraHS - QoL questionnaire in 6 and 12 months after simultaneous hernioplasty. Ukrainian herniologists also use the EuraHS - QoL questionnaire to determine the quality-of-life outcomes after hernioplasty [10, 11]. This questionnaire consists of 3 domains: 1) pain in the area of the hernia; 2) limitation of movements due to pain or discomfort in the area of the hernia; 3) cosmetic discomfort (discomfort due to the appearance of the abdomen and hernia areas). There are a total of 9 questions in the questionnaire, each of which is evaluated on a 10-point

scale with a maximum score of 90 ("0" - no pain, restriction of movement and discomfort; "10" - the most severe pain, restriction of movement and discomfort). The EuraHS - QoL questionnaire is completed before and after surgery at certain time periods determined by the researchers.

The results of the survey of 32 patients after 36 simultaneous hernioplasty are presented in Table VI. When conducting a survey of patients who underwent simultaneous inguinal pre-peritoneal hernioplasty, questionnaires were filled out for each hernioplasty (36 questionnaires were received).

According to Table VI, the quality-of-life outcomes after the one-stage prostatectomy accompanied with hernioplasty in 6 and 12 months were statistically better than before these operations. Thus, after analyzing the long-term (more than 1 month after the operation) postoperative results in patients with benign hyperplasia and inguinal hernia, it was established that the quality-of-life improved. There were no long-term postoperative complications of the main and simultaneous operations too.

Prospects for further development are the screening of benign prostatic hyperplasia in men with inguinal hernias in surgical hospitals followed by the planning of one-time surgical correction of these pathologies with the involvement of a urologist.

CONCLUSIONS

Performing a one-stage prostatectomy accompanied with hernioplasty in patients with benign prostatic hyperplasia and hernia inguinalis does not worsen the immediate and long-term results of operation, instead, it helps to eliminate two diseases at the same time from one surgical approach.

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ANATOMICAL AND BIOMECHANICAL ROLE OF STATIC STABILIZERS OF THE ACROMIOCLAVICULAR JOINT

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ABSTRACT

The aim: To determine the anatomical and biomechanical significance of the static stabilizers of the acromioclavicular joint by conducting numerical modeling using the finite element method and experimental investigation

Materials and methods: To ensure the study, modeling of the deformation processes of the clavicle and scapula systems with various combinations of ligament damage was conducted. The COMPAS-3D software package was used to build the simulation model, which allowed obtaining models that are closest to reality. To verify the results of the numerical modeling, corresponding studies of the mechanical characteristics and determination of the stiffness of the investigated systems were carried out using the upgraded TIRAtest-2151 testing stand

Results: The stiffest system is the system in which all ligaments are intact, and the sequence of decreasing stiffness of the system is presented in the following order: damage to lig. trapezoideum; lig. conoideum; lig. claviculo-acoacromiale inferior; lig. claviculo-acoacromiale superior; the coracoclavicular ligament complex; the acromioclavicular ligament complex

Conclusions: Static stabilizers in general, and their components in particular, are characterized by significant anatomical and functional features. The natural stabilization of the acromioclavicular joint is provided by their synergistic interaction, which is the basis for the development and implementation of surgical interventions, the scope of which includes the restoration of both ligament complexes. The loss of stiffness in the «clavicle-scapula» system is significantly more pronounced when lig. acromioclavicular superior and inferior are damaged (8.5 N/mm) than when lig. conoideum and lig. trapezoideum are damaged (11.6 N/mm)

KEY WORDS: Acromioclavicular joint, static stabilizers, biomechanical

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INTRODUCTION

Acromial end of the clavicle dislocations (AEC) make up from 6.8% to 26.1% of all dislocations, and rank third after shoulder and forearm dislocations. In the context of acute traumatic injuries affecting the shoulder girdle region, it is noteworthy that the incidence of AEC dislocations exceeds 12%. These injuries are more common in working-age men (30 to 40 years old) and athletes who engage in contact sports [1].

The acromioclavicular joint (ACJ) possesses six degrees of freedom in the anterior-posterior and superior-inferior planes. The rotational range of motion is approximately 45°, with vertical motion up to 15°, sagittal motion up to 19°, and frontal motion up to 29°. The proper anatomical relationship between the flat and small joint ends of the clavicle and the acromion process of the scapula, as well as the primary stabilization of the joint during movement, is ensured through the dynamic and static stabilizers. Crucial anatomical

structures that contribute to the static stabilization of the joint include the joint capsule, acromioclavicular and coracoclavicular ligaments [2].

An examination of the relevant literature reveals an ongoing debate spanning nearly a century regarding the primary stabilizer of the acromioclavicular joint (ACJ), which is commonly referred to as the “key” component in this regard. At present, two primary positions have been established: (1) the claviculo-acromial ligament serves as the primary stabilizing structure, and (2) the coracoclavicular ligament assumes the dominant role in joint stabilization.

The aforementioned debate has paved the way for a multitude of surgical approaches to the restoration of damaged ligamentous complexes, with over 150 methods currently proposed. The sheer volume of suggested techniques was intended to promote an individualized approach to the treatment of AEC dislocations. Nevertheless, the lack of clear indications

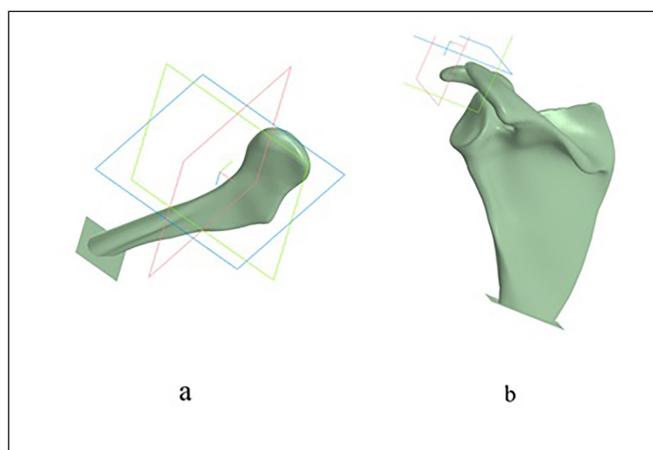


Fig. 1. Simplified models of the studied system: a) - clavicle, b) – scapula

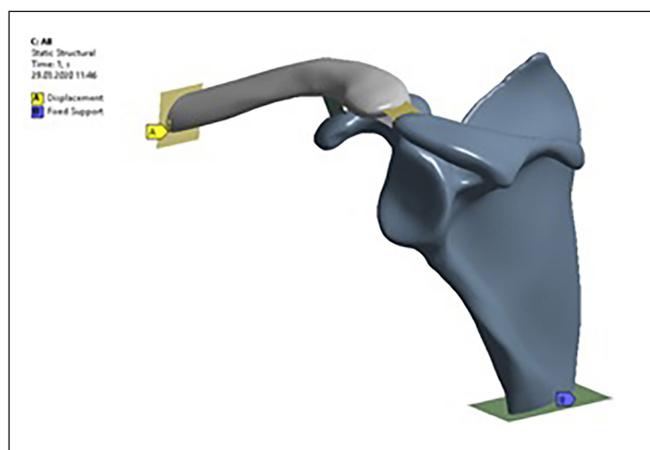


Fig. 2. Investigated system

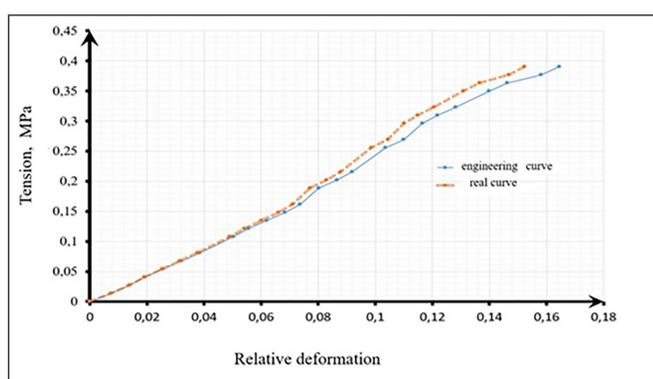


Fig. 3. Experimental deformation curves of the rubber bands.

for their employment has compromised this direction.

The use of different surgical techniques for treating AEC dislocations allows for an average of final outcome measures, among which negative results account for 9 to 12%. The relatively high rate of unsatisfactory outcomes is due to several main factors, such as horizontal instability of the AEC, loss of congruency within the joint, heterotopic ossification, and post-traumatic ACJ osteoarthritis [3].

Given the current challenges and inconsistencies in treating ACJ dislocations, further research, improvement, and development of novel treatment approaches are objectively warranted.

THE AIM

To determine the anatomical and biomechanical significance of the static stabilizers of the acromioclavicular

joint by conducting numerical modeling using the finite element method and experimental investigation.

MATERIALS AND METHODS

The feasibility of determining and comparing the stiffness of the clavicle-scapula connection systems depending on the combination of damaged ligaments investigated by this method is justified due to the fact that conducting in-vivo experimental studies to determine the stiffness of such systems is not always possible due to their complexity, ethical considerations, and labor-intensive nature. It is also important to consider that the characteristics of the ligamentous apparatus depend on the time since death.

In order to facilitate the investigation, computer-based simulations of the deformation processes of the clavicle (C) and scapula (S) systems with varying damage combinations were conducted.

The software package KOMPAS-3D was utilized to construct an accurate simulation model of C and S, taking into consideration their anatomical peculiarities. Subsequently, the presented models of the clavicle and scapula were incorporated into a final model, taking into account the actual experimental studies' method of fixation and loading (Fig 1a, b).

We made a numerical simulation of the deformation process of the C and S systems with different combinations of damaged ligaments, using the Ansys Workbench numerical package and the static structural module. The investigated system with all intact ligaments is presented in Fig 2.

Table I. Stiffness of systems with different combinations of damaged ligaments, obtained through numerical modeling.

Case	a	b	c	d	e	f	g
Stiffness, N/mm	19.5	15.1	16	13	14.8	8.5	11.6

Note: a - intact ligamentous apparatus; b - damage to lig. conoideum; c - damage to lig. trapezoideum; d - damage to lig. claviculo-acromiale superior; e - damage to lig. claviculo-acromiale inferior; f - damage to the acromioclavicular ligament complex; g - damage to the coracoclavicular ligament complex.

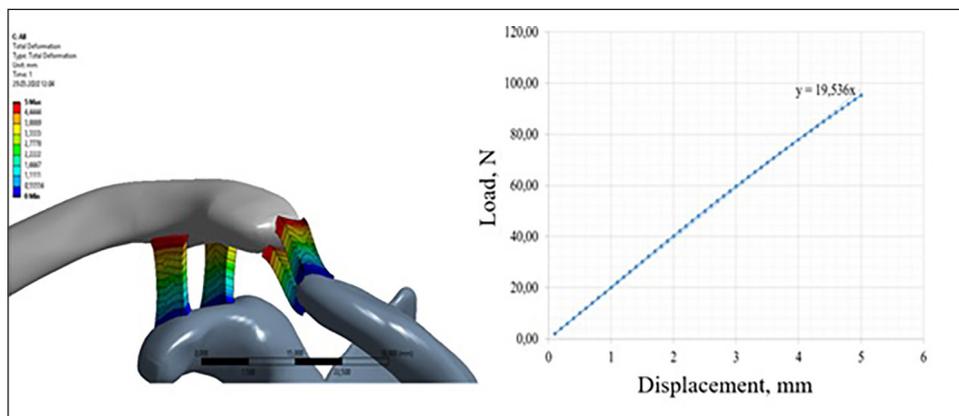


Fig. 4. The model of static ASJ stabilizers and the relationship between the applied force and displacement under standard conditions

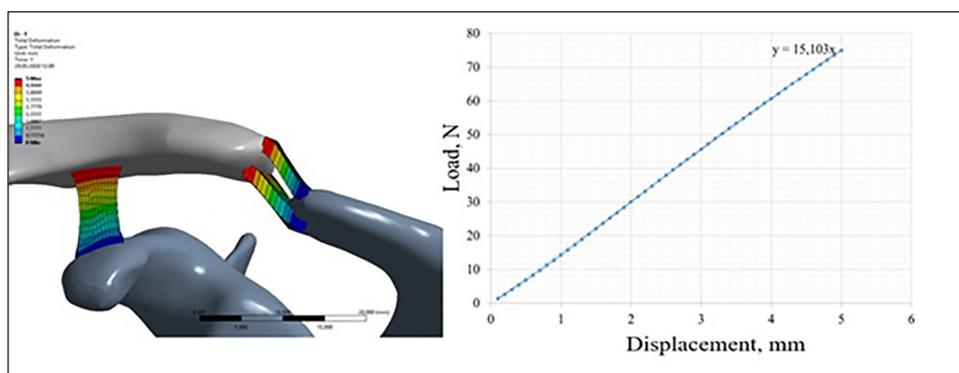


Fig. 5. The model of static stabilizers of the ACJ and the relationship between the applied force and displacement under damage to the lig. Conoideum

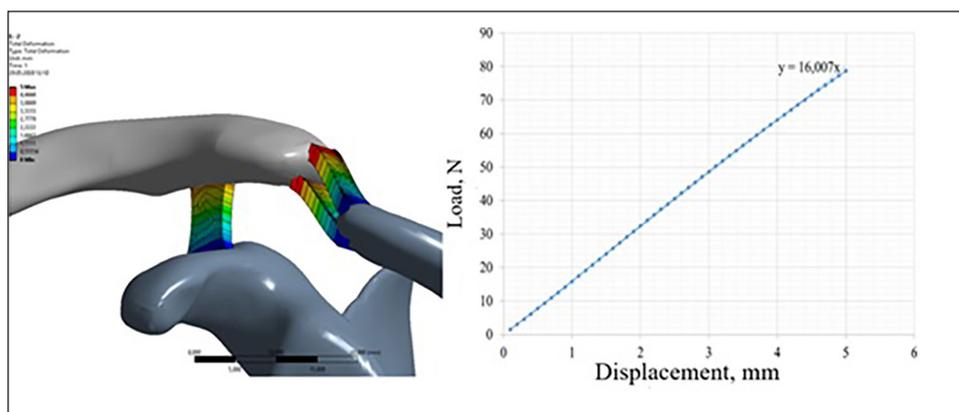


Fig. 6. Model of static stabilizers of the ACJ and the relationship between the applied force and displacement under damage to the ligament trapezoideum

In the analysis, the clavicle (C) and scapula (S) were treated as rigid bodies with infinite stiffness, which simplified the computations. This assumption was based on the observation that the stiffness of C and S is significantly greater than that of the ligaments, and, therefore, the impact of the ligaments on the system's behavior could be neglected under relatively low loads.

Medical rubber bands were used as ligaments in the experimental investigations. This material was selected due to the Neo-Hookean model, which is based on the stress-strain curve obtained from experimental research (Fig 3). This material is highly elastic and appropriate for use as ligaments in the investigated system.

To verify the results of numerical modeling, relevant investigations of mechanical characteristics and determination of the stiffness of the studied systems

were conducted, using the modernized testing stand TIRAtest-2151.

The boundary conditions were selected according to real experimental studies, so that the lower part of S was rigidly fixed, similar to fixing the sample to the frame of the testing machine. Displacements of 5, 10, and 12 mm were applied to part C in the corresponding plane, according to the load applied during the experiment.

RESULTS

The model of the static ASJ stabilizers and the numerical simulation results, with the relationship between applied force and displacement under standard conditions are presented in Fig 4.

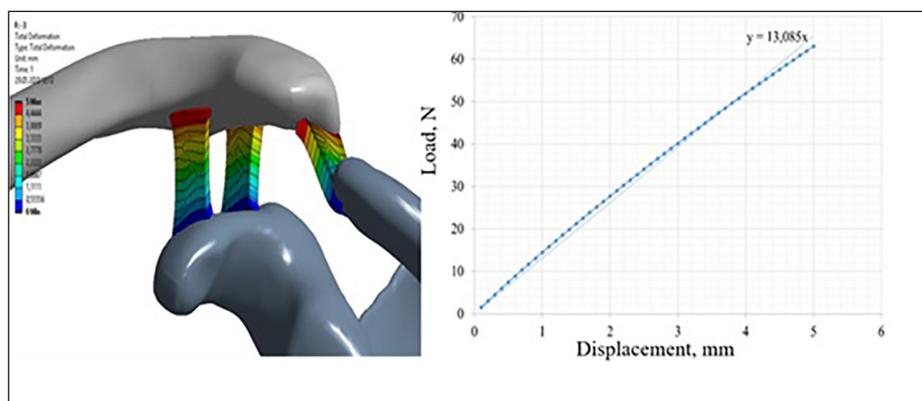


Fig. 7. The model of static stabilizers of the AC joint and the relationship between the applied force and displacement under damage to the superior acromioclavicular ligament.

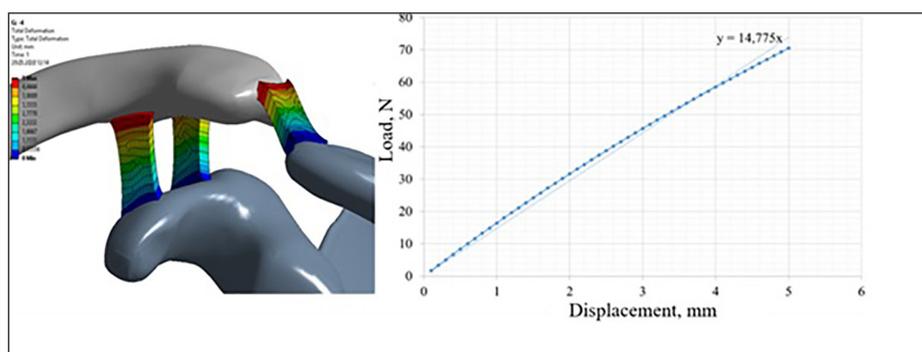


Fig. 8. The model of static stabilizers of the ACJ and the relationship between the applied force and displacement under damage to the ligamentum claviculo-acromiale inferius

Numerical modeling was carried out for the present research system to obtain displacement distribution fields with different combinations of damaged connections.

The model of static stabilizers of the ACJ and the results of the dependence of the applied force on the displacement in the case of damage to the ligamentum conoideum are presented in Fig 5.

The model of the static stabilizers of the AC joint and the dependence of the applied force on displacement with ligamentum trapezoideum damage is shown in Fig 6.

The model of the static stabilizers of the ACJ and the relationship between the applied force and displacement under damage to the lig. claviculo-acoacromiale superior is presented in Fig 7.

The model of static stabilizers of the ACJ and the relationship between the applied force and displacement under damage to the ligament claviculo-acromiale inferior is presented in Fig 8.

The model and the relationship between the applied force and displacement under complete damage of the acromioclavicular ligament complex are presented in Fig 9.

Model and relationship between the applied force and displacement under complete damage of the clavicular coracoid ligament complex is shown in Fig 10.

Considering the presented results and obtained dependencies, the stiffness values for the respective systems are presented in the form of Table I.

Based on the results presented in Table I, it can be concluded that the stiffest system is the system in case

(a), where all ligaments are intact. The decreasing order of system stiffness is as follows: a, c, b, e, d, g, f.

Therefore, the results indicate that the loss of stiffness in the “clavicle-scapula” system is significantly more pronounced in case of damage to the ligaments lig. acromioclaviculare superior and inferior (8.5 N/mm) compared to lig. conoideum and lig. trapezoideum damage (11.6 N/mm).

DISCUSSION

An examination of the relevant literature reveals an ongoing debate spanning nearly a century regarding the main stabilizer of the AC joint: is it the coracoclavicular ligament (CCL) or the acromioclavicular ligament (CAL)?

Volkovich N.M. (1928), as well as Buason and Ader (1930), were among the first to conduct experimental research on cadavers to determine the role of the ACJ ligamentous complex in maintaining congruity. When the CAL was severed, the researchers noted vertical instability, whereas when the CCL was incised, there was a significant displacement of the clavicle relative to the acromial process of the scapula (greater than 2 cm). The results led to the conclusion that isolated CAL damage leads to subluxation, while combined damage involving both CAL and CCL results in ACJ dislocation [4, 5].

D. Muscolo conducted an experiment on cadavers where he damaged ligaments and conducted radiographic studies to assess the relationship in the ACJ.

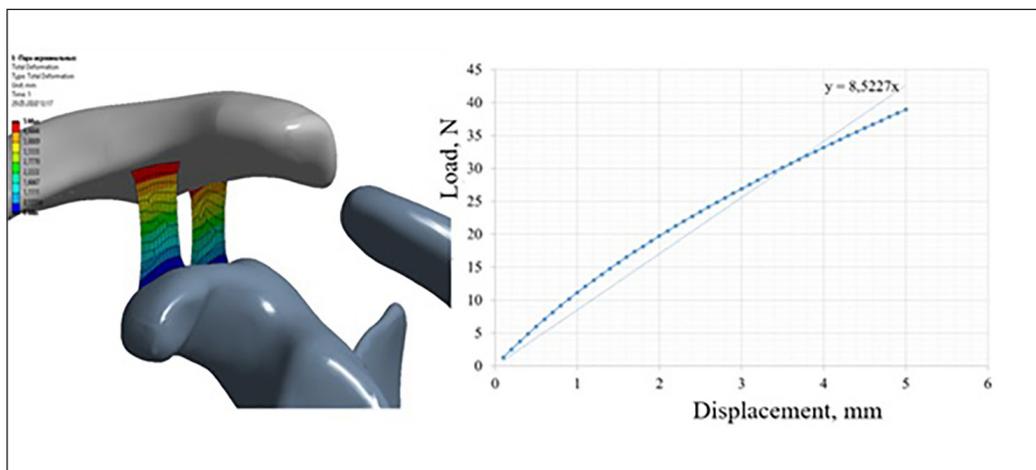


Fig. 9. Model and relationship between the applied force and displacement under complete damage to the acromioclavicular ligament complex

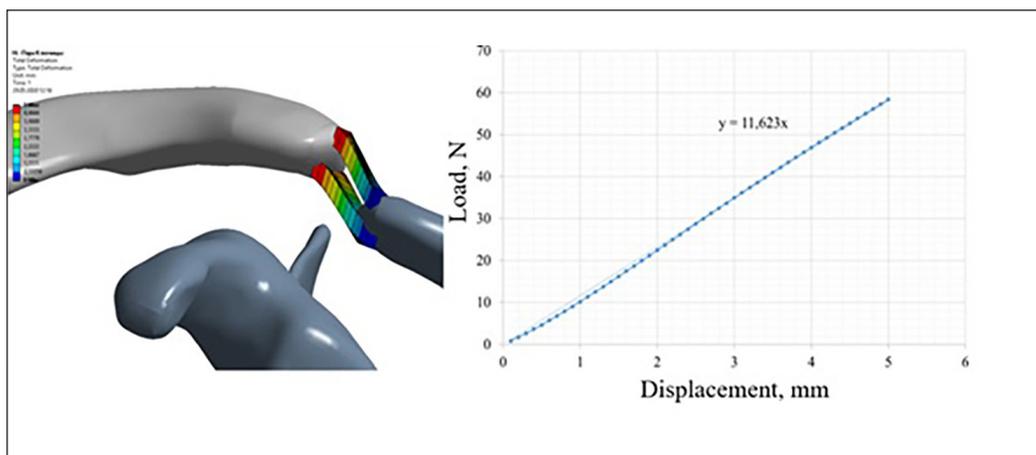


Fig. 10. Model and relationship between the applied force and displacement under complete damage to the acromioclavicular ligament complex

He observed only minor congruity disturbance on the radiograph with loading after severing the CCL, whereas cutting the CAL resulted in a dislocation. This led him to conclude that the CAL plays a leading role in stabilizing the ACJ [6].

R. Urist (1963) and M. Rosenorn and V. Pedersen (1974) also found that severing the CCL does not cause dislocation of the ACJ [7].

On the other hand, an opposing view highlights the crucial role of the coracoclavicular complex.

K. Dohn (1956) found that the strength of CAL is 36-46 kgf, whereas CCL exceeds 80 kgf, and its restoration is an essential component in stabilizing the ACJ [8].

Other researchers have also emphasized the significance of the coracoclavicular ligament in ACJ stabilization [9].

The desire to determine the primary ligamentous complex responsible for stabilizing the ACJ not only serves as the basis for an ongoing debate, but also forms the groundwork for the development and implementation of surgical techniques aimed at restoring the ACJ ligamentous apparatus. Two approaches are currently used for this purpose: restoration of the CC ligament complex [10-12], or the CA ligament complex [13,14].

Anatomical and biomechanical research continues to deepen our understanding of these structures.

Nakazawa detailed the morphology of the acromioclavicular ligament, specifically its bipartite arrangement, through histological and anatomical studies. In addition, a clear structural advantage of the superoposterior bundle (SPB) over the less consistent anteroinferior bundle (AIB) was also found. The SPB is a well-defined capsular thickening that is consistently present in all samples at a 30° orientation. Its attachments originate from the upper, posterior, and lower portions of the clavicle [15].

Ausberto Velasquez Garcia et al. conducted thorough research on the functional role of these components and concluded that although the two bundles of the ACJ ligament function in a complementary mode to maintain the kinematics, the AIB plays the primary role in joint constraint throughout the shoulder motion examined. Furthermore, the SPB appears to help avoid excessive anterior and superior translation, particularly during horizontal adduction [16].

Modern anatomical studies have revealed a unique orientation of the CAL portions, the attachments of the lig. conoideum and lig. trapezoideum on the procesus coracoideus and C, indicating their diverse function.

However, these structures only provide natural stabilization of the ACJ through their combined action [17].

Practice confirms this important concept. An anatomical reconstruction of the CCL demonstrates better primary stability similar to native ligaments compared to non-anatomical reconstruction. However, in 42% of cases, persistent dynamic posterior instability, which is considered as a postoperative deficit of the ACL, was detected [18-20].

Further clinical and anatomic-biomechanical studies indicate that isolated restoration of the acromioclavicular or coracoclavicular ligament complex does not provide complete stability of the ACJ and demonstrate the necessity of ligament reconstruction of both locations [21,22].

CONCLUSIONS

1. Static stabilizers in general and their components in particular are characterized by significant anatomical and functional peculiarities. Natural stabilization of the acromioclavicular joint is provided by their synergistic interaction, which is the basis for the development and implementation of surgical interventions, the scope of which includes the restoration of both ligament complexes.
2. Loss of stiffness in the "clavicle - scapula" system is significantly more pronounced in case of damage to the superior and inferior acromioclavicular ligaments (8.5 N/mm) than in case of damage to the conoid and trapezoid ligaments (1.6 N/mm).

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

EVALUATION OF THE ANTIHYPERTENSIVE EFFECT OF THE THYME AMONG PATIENTS SUFFERING FROM HYPERTENSION IN IRAQ

DOI: 10.36740/WLek202312110

Qayssar Joudah Fadheel¹, Rusul Ahmed Mohammed²¹DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, UNIVERSITY OF BABYLON, IRAQ²DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY, COLLEGE OF PHARMACY, AL MUSTAQBAL UNIVERSITY, IRAQ**ABSTRACT**

The aim: of research is to test effectiveness of thyme on patients with high blood pressure and comparing between blood pressure of patients who took thyme and patients who did not among different ages.

Materials and methods: This study concerning patients with high blood pressure, (3/2022 to 3/2023). Collecting data was depend on interviewed patients directly to get information. A questionnaire paper includes several questions. Interview included convincing patients to take thyme for 15 days to know how could thyme effect on their blood pressure and over different patients.

Results: A significant difference in distribution between male and female within control and treated groups. A significant difference between all age groups when compared control and treated group, and decrease in systolic blood pressure when patients taking own drug plus thyme compared with patients taking own drug alone, but non-significant decrease in systolic blood pressure when compared with control group, a non-significant decrease in systolic blood pressure in patients taking own drug when compared with control group. A significant decrease in diastolic blood pressure when patients taking own drug plus thyme in comparison with patients taking own drug alone, but non-significant reduction in diastolic blood pressure when compared with group which considered control, non-significant decrease in diastolic blood pressure in patients taking own drug when compared with control group.

Conclusions: Thyme herbs induce normalization hypertension and keeps blood pressure in usual range. The available clinical trial data provide evidence for the safe utilization of herbal preparations containing Thyme in the treatment of hypertension.

KEY WORDS: hypertension, thyme, carvacrol, rosmarinic, herb

Wiad Lek. 2023;76(12):2614-2624

INTRODUCTION

Hypertension is characterized by the presence of consistently elevated blood pressure in the systemic arteries. Blood pressure (BP) is frequently quantified as the ratio between systolic pressure and diastolic blood pressure (the pressure during relaxation of the heart). The term hypertension, referred to a systolic blood pressure (SBP) greater than 140 mmHg or a diastolic blood pressure (DBP) greater than 90 mmHg, is a significant and escalating health concern on a global scale [1]. Cardiovascular disease is highly prevalent among individuals aged 60 years or older, with nearly two-thirds of this population being affected by this condition [2]. According to estimates, uncontrolled hypertension is attributed to approximately 7.5 million global fatalities annually [3]. In the United States, this condition alone incurs health-care expenditures, medication costs, and productivity losses amounting to over 47 billion dollars [4, 5].

The term "essential hypertension" is used to describe the condition in approximately 95% of hypertensive patients, where elevated blood pressure is a consequence of intricate interactions between various environmental and genetic factors. Conversely, about 5% of patients experience hypertension due to identifiable specific causes, referred to as secondary hypertension [6]. Lifestyle modifications for hypertension encompass various strategies, such as weight reduction for patients who are obese or overweight, embracing the Dietary Approaches to Stop Hypertension (DASH) eating plan, limiting dietary sodium intake to an ideal target of 1.5 grams per day (equivalent to 3.8 grams of sodium chloride), engaging in regular aerobic physical activity, practicing moderation in alcohol consumption (limiting intake to two or fewer drinks per day), and discontinuing smoking. For the majority of patients with prehypertension, lifestyle modification is considered to



Fig. 1. Thyme plant.

be an adequate approach. However, for hypertensive patients who also have further cardiovascular risk factors or damage to the target organ, lifestyle changes alone are not enough [7]. Several studies have demonstrated that thyme exhibits a positive impact on the reduction of blood pressure. The selection of an initial drug is obtained by blood pressure level elevation and the available of compelling indications for specific medications. The selection of pharmaceutical agents for the treatment of hypertension (HTN) is influenced by several factors, including but not restricted to age, presence of comorbidities, ethnicity, and pregnancy status. The primary medication classes consist of calcium channel blockers (CCBs), angiotensin-converting enzyme (ACE) inhibitors, angiotensin II receptor blockers (ARBs), and thiazide diuretics, which have been identified as appropriate first-line treatment choices [8].

THYME

Thyme, a fragrant perennial evergreen herb, is classified under the genus *Thymus* within the *Lamiaceae* family (Fig. 1). It is characterized by its production of petite flowers that can range in color from white to lilac or pink. The existence of more than 350 thyme species can be attributed primarily to their high propensity for hybridization. Thyme is widely recognized for its abundant array of therapeutic advantages. Thyme has been historically utilized for its therapeutic properties, with its leaves, flowers, and oil being employed in the treatment of diverse diseases and ailments. Thyme possesses both

antiseptic and antimicrobial properties, rendering it a highly efficacious remedy for treating colds, coughs, and various respiratory ailments. Thyme is known to exhibit antibacterial properties, making it a common ingredient in facial cleansers and anti-acne creams. Thyme possesses a bioactive compound that exhibits potential in reducing blood pressure levels. Thyme, akin to other herbal remedies, exhibits a limited number of adverse effects. Thyme possesses bioactive constituents, namely thymol and carvacrol, which have the potential to induce irritation of the mucous membranes in individuals exhibiting sensitivity. There is a lack of established empirical evidence regarding the safety of this herb for pregnant and breastfeeding women, as well as a dearth of contraindications. Nevertheless, due to thyme's historical use as a traditional method to stimulate menstruation, there exists a potential risk of miscarriage for pregnant women [9].

THE AIM

The aim of this research is to test effectiveness of thyme on patients with high blood pressure and comparing between blood pressure of patients who took thyme and patients who did not among different ages.

MATERIALS AND METHODS

This study concerned patients with high blood pressure, and it was conducted during the period from November 2022 to March 2023, during which

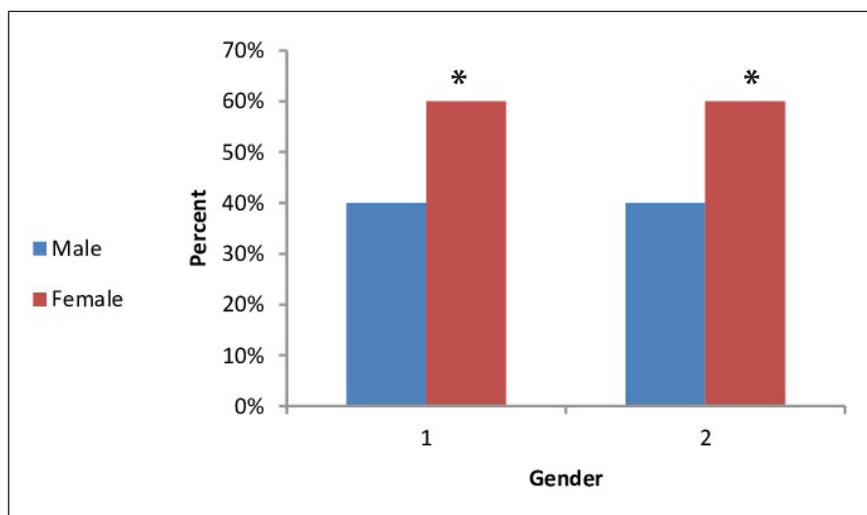


Fig. 2. Gender distribution among study population (* - significant difference).

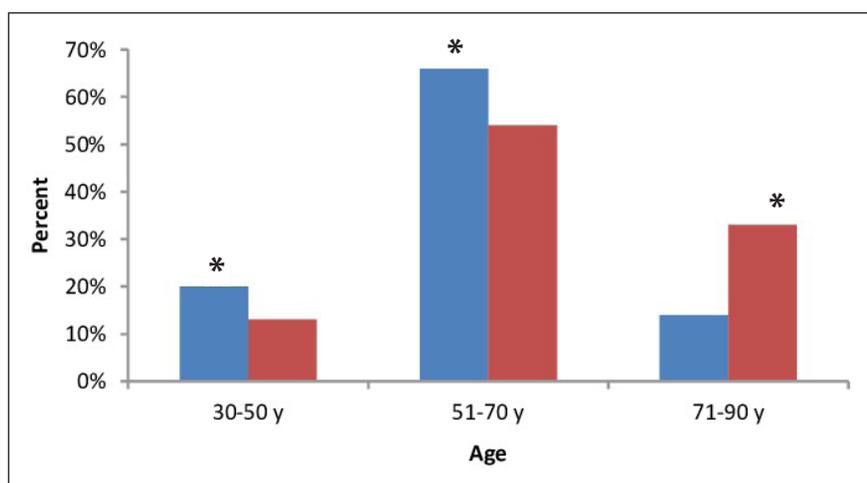


Fig. 3. Age distribution among study population (* - significant difference).

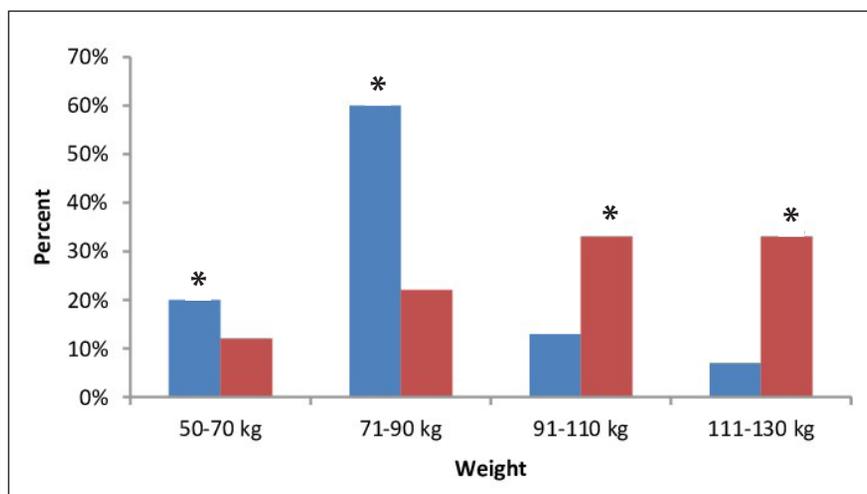


Fig. 4. Weight distribution among study population (* - significant difference).

we did a field research over people with high blood pressure that we know, and who include people from our family and friends, and also people we have a relationship with them , so there was no need to go To the Hospital to look for patients, because we had to follow up the patients closely and measure the extent of their blood pressure changes to obtain the better result.

INCLUSION CRITERIA

Included patients with high blood pressure who controlled this high by taking various antihypertensive drugs.

EXCLUSION CRITERIA

Patients who have high blood pressure and do not take any medication to control it and control

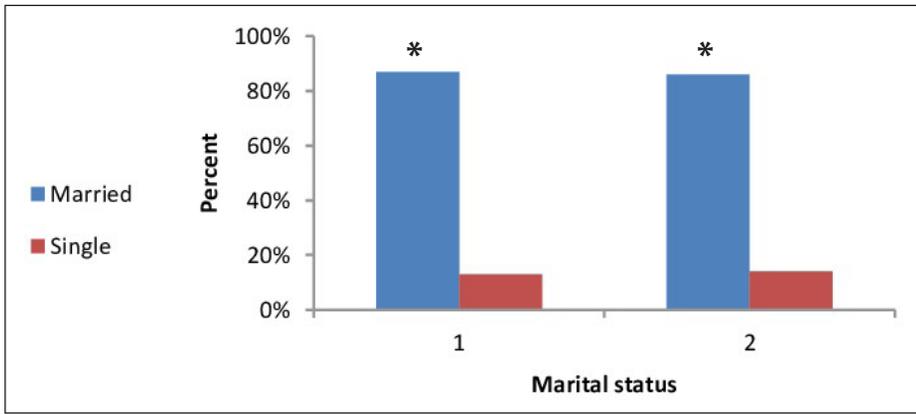


Fig. 5. Distribution of marital status among study population (* - significant difference).

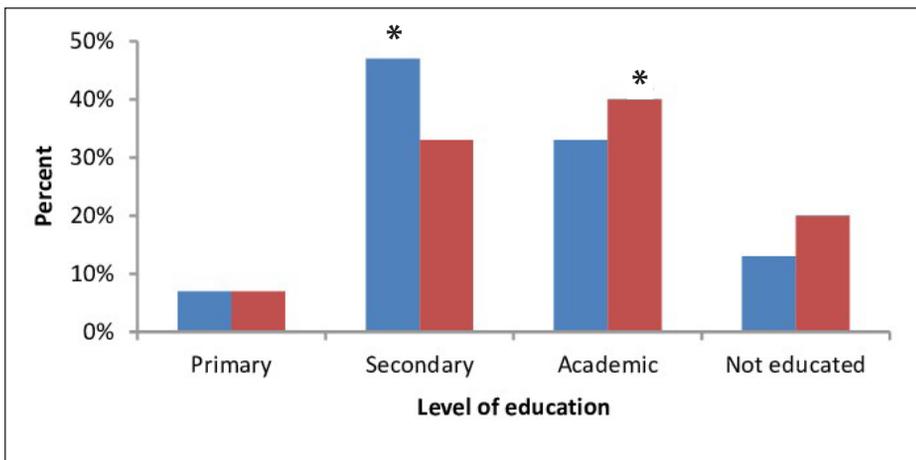


Fig. 6. Distribution of level of education among study population (* - significant difference).

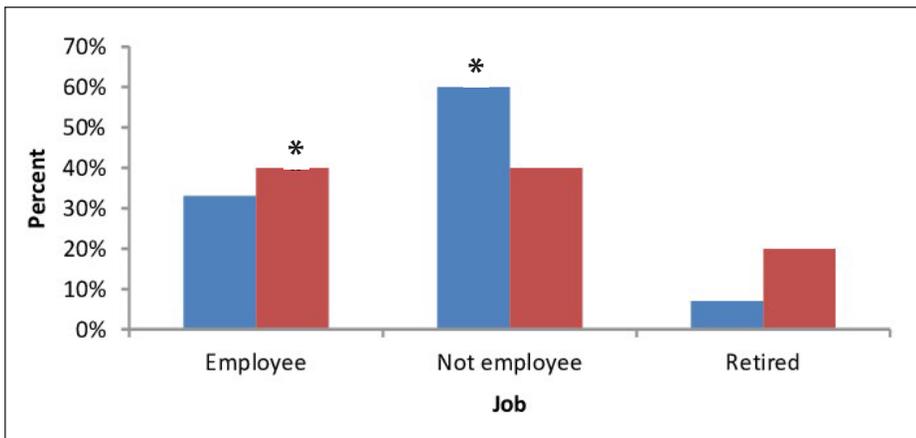


Fig. 7. Distribution of job among study population (* - significant difference).

it through diet and physical activity only, patients who did not complete the treatment and did not keep in touch with us, and patients who refused to take thyme.

SAMPLING

This clinical trial includes 30 patients with hypertension, 15 patients of them taking thyme to test its effect on lowering hypertension and the other 15 didn't take thyme using as control group patients to compare between their results.

COLLECTION OF DATA

The method of data collection was depended on interviewed patients directly (personal) to get several information about them. A questionnaire paper using for this direct interview and the paper include several questions about (patients age, marital status, their level of education, job, place of residency, duration of hypertension and their diet and life style and the medication that taken by them). This direct interview also included convincing patients to take thyme for 15 days to know how could the thyme effect on their blood pressure and over different patients.

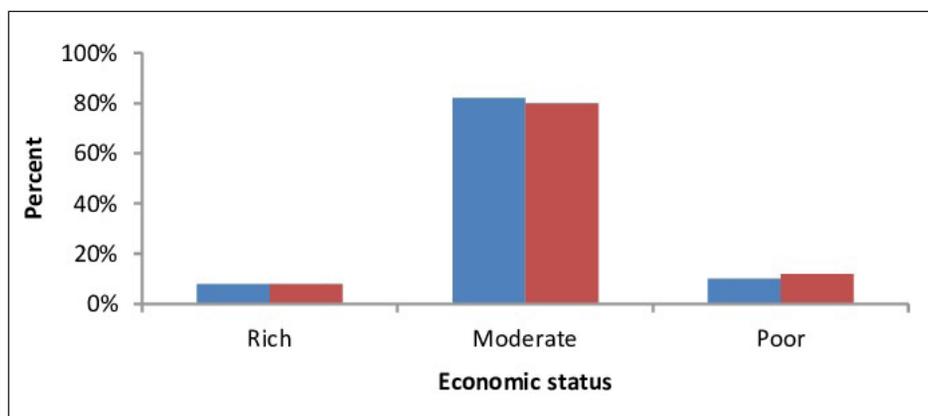


Fig. 8. Distribution of economic status among study population (* - significant difference).

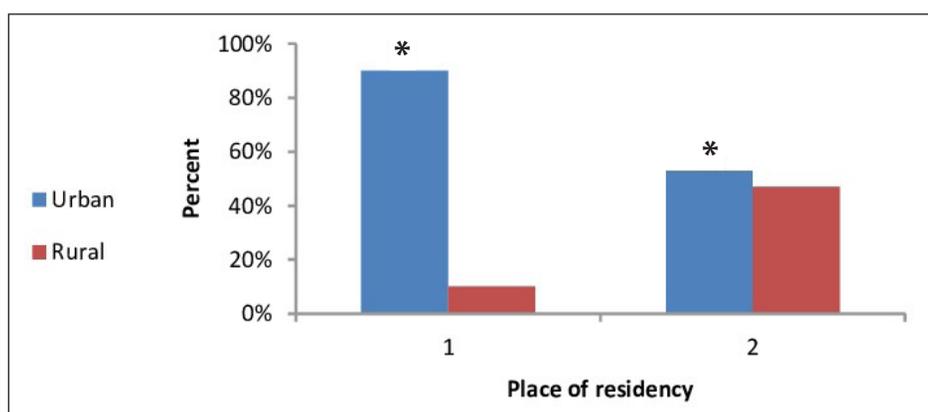


Fig. 9. Distribution of place of residency among study population (* - significant difference).

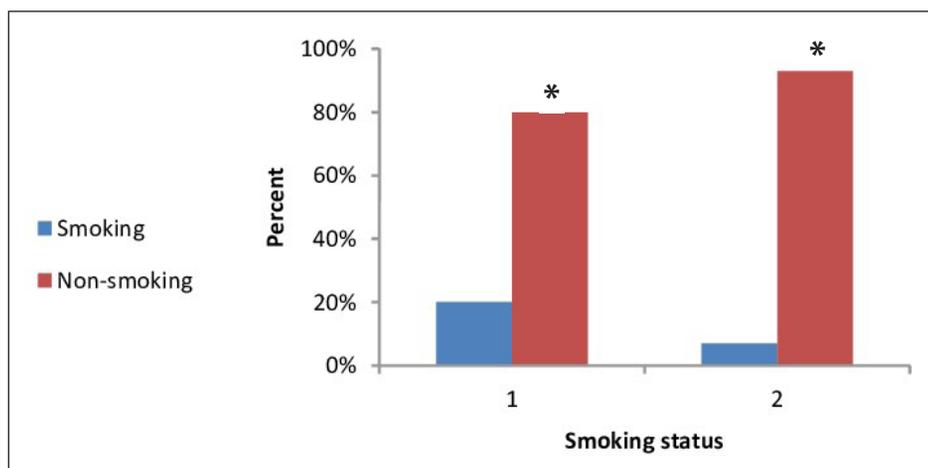


Fig. 10. Distribution of smoking status among study population (* - significant difference).

STATISTICAL ANALYSIS

The statistical analysis was conducted using SPSS 16.0. Consultation was sought from a statistical expert for the test employed. The study involved comparing the differences between each pair using paired-sample analysis. The application of the student's t-test was facilitated through the utilization of Microsoft Office Excel 2010. A P-value below 0.05 was deemed to be statistically significant in all conducted tests.

RESULTS

EFFECT OF GENDER DISTRIBUTION AMONG STUDY POPULATION

The present study reveals a significant difference in distribution between male and female within the control group and within treated group, but non-significant differences between control and treated group as shown in (Fig. 2).

EFFECT OF AGE DISTRIBUTION AMONG STUDY POPULATION

The current findings show a significant difference between age (30-50 years) as compared with age (51-70 years) but non-significant differences between age (30-50 years) as compared with age (71-90 years), in addition to that there was a significant difference between

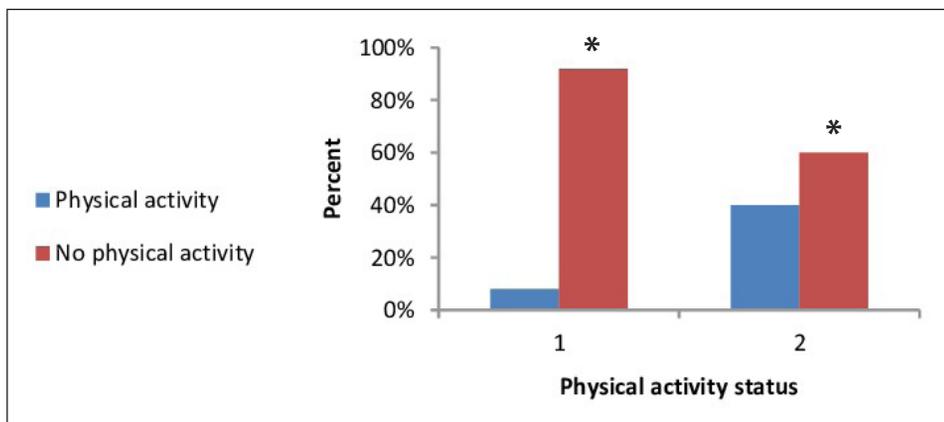


Fig. 11. Distribution of physical activity status among study population (* - significant difference).

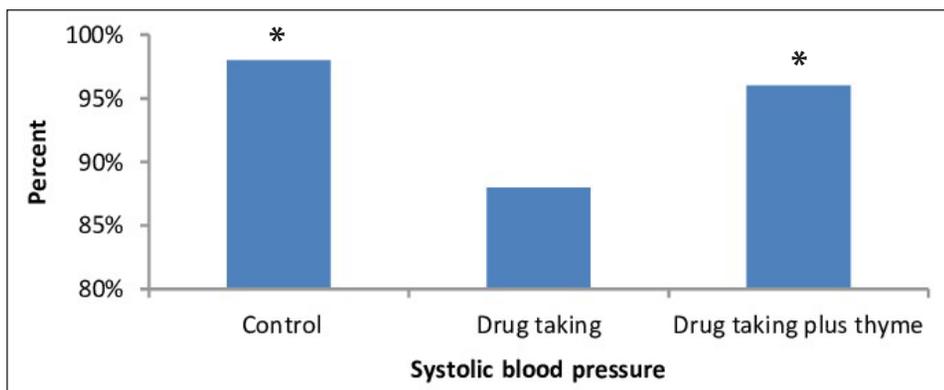


Fig. 12. Distribution of systolic blood pressure among study population (* - significant difference).

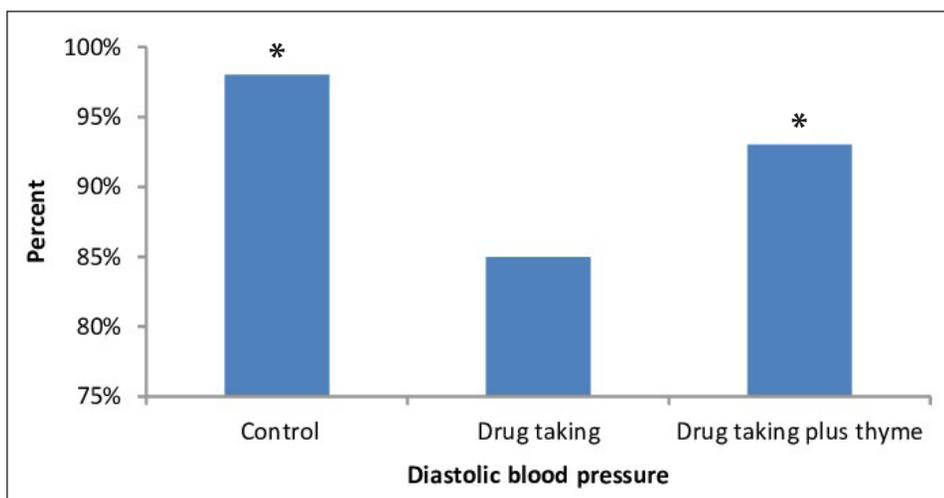


Fig. 13. Distribution of diastolic blood pressure among study population (* - significant difference).

age (51-70 years) as compared with age (71-90 years) within the control group, also there was a significant difference between age (30-50 years, 51-70 years, 71-90 years) within treated group. There was a significant difference between all age groups when compared control and treated group (Fig. 3).

EFFECT OF WEIGHT DISTRIBUTION AMONG STUDY POPULATION

The current study reported that there was a significant difference between weight (71-90 kg) as compared with weight (50-70 kg, 91-110 kg, 111-130 kg), also

significant differences between weight (50-70 kg) as compared with weight (111-130 kg), but non-significant differences between weight (91-110 kg) as compared with weight (111-130 kg) within the control group. There were non-significant differences between weight (91-110 kg) as compared with weight (111-130 kg), but significant differences between weight (91-110 kg, 111-130 kg) as compared with weight (50-70 kg, 71-90 kg), in addition to those non-significant differences between weight (50-70 kg) as compared with weight (71-90 kg) within the treated group. There was a significant difference between all weight groups when compared control and treated groups (Fig. 4).

EFFECT OF MARITAL STATUS DISTRIBUTION AMONG STUDY POPULATION

The present study shows a significant difference between married and single population within control and treated groups, also there was a significant difference between married and single population between control and treated groups (Fig. 5).

EFFECT OF LEVEL OF EDUCATION DISTRIBUTION AMONG STUDY POPULATION

The current study reported that there was a significant difference between primary, secondary, academic, and uneducated population within control and treated groups. There were non-significant differences between primary level of education when compared control group with treated group, but significant differences between secondary, academic, and uneducated population when compared control group with treated group (Fig. 6).

EFFECT JOB DISTRIBUTION AMONG STUDY POPULATION

The findings of study show a significant difference between employee, not employee, and retired within control group. Also, there was significant differences between retired as compared with employee and not employee but non-significant differences between employee and not employee within treated group. There was a significant difference between not employee and retired but non-significant differences between employee when compare of the control and treated groups (Fig. 7).

EFFECT ECONOMIC STATUS DISTRIBUTION AMONG STUDY POPULATION

The current study shows that there was a significant difference between moderate economic status as compared with poor and rich within both control and treated groups, but non-significant differences between poor, rich, and moderate economic status when compare of the control and treated groups (Fig. 8).

EFFECT PLACE OF RESIDENCY DISTRIBUTION AMONG STUDY POPULATION

The findings of present study reported a significant difference between rural and urban distribution of study population within control and treated groups,

also a significant difference when compared control and treated groups (Fig. 9).

EFFECT SMOKING STATUS DISTRIBUTION AMONG STUDY POPULATION

The findings of present study recorded a significant difference between smoking and non-smoking population within control and treated groups and between control group as compared with treated group (Fig. 10).

EFFECT OF PHYSICAL ACTIVITY STATUS DISTRIBUTION AMONG STUDY POPULATION

The results of present study show a significant difference between physically active and non-physically active population within control and treated groups and between control group as compared with treated group (Fig. 11).

EFFECT OF TREATMENT ON SYSTOLIC BLOOD PRESSURE

The findings of present study reveal a significant reduction in systolic blood pressure when patients taking own drug plus thyme in comparison with patients taking own drug alone as in figure 12.

EFFECT OF TREATMENT ON DIASTOLIC BLOOD PRESSURE

The current study shows a significant reduction in diastolic blood pressure when patients taking own drug plus thyme in comparison with patients taking own drug alone (Fig. 13).

DISCUSSION

In the present study of 30 hypertension patient participants aged 35 years and older, the results confirm that a significant decrease in blood pressure in patients who took thyme plus to their own medication comparing to patients taking own medications only, Thyme contains powerful compounds, rosmarinic acid is one such compound, rosmarinic acid is a natural compound that has vasorelaxant effect. Rosmarinic acid has been found to exhibit efficacy in the reduction of blood pressure. Currently, the management of blood pressure involves a combination of medical interventions and the adoption of a health-conscious lifestyle. Nevertheless, scholarly research indicates that alternative treatment modalities are frequently employed in conjunction with conven-

tional medical interventions [10]. The present study reveals a significant difference in distribution between male and female within the control group and within treated group, but non-significant differences between control and treated group (Fig. 2). This result is in consistency with the result of study [11, 12], which stated that in China, where prevalence of hypertension is observed to be higher among women compared to men, with rates of 51.9% and 42.5% respectively. Similarly, the rates of treatment and control of hypertension are also higher among women, with percentages of 46.6% and 35.6% respectively. Furthermore, when considering the usage of antihypertensive drugs, there is no significant difference in the control rates between men and women, both at 37.0% and 38.0% respectively. The data indicates that the prevalence of hypertension was found to be higher in men compared to women, with rates of 24.5% and 21.9% respectively.

The Renin-Angiotensin System (RAS) is widely recognized as a pivotal mechanism underlying gender disparities in the development and regulation of hypertension. Furthermore, it is acknowledged that sex hormones exert a significant influence on blood pressure (BP) regulation. Multiple studies have demonstrated that both endogenous and exogenous estrogens have the capacity to decrease blood pressure levels in postmenopausal women who have hypertension [13-15]. There was a significant difference between age (30-50 years, 51-70 years, 71-90 years) within treated group. There was a significant difference between all age groups when compared control and treated group (Fig. 3). This result is in consistency with the result of the study [16], which states that the general population that systolic and diastolic blood pressure increase with age and that systolic blood pressure continues to increase after the seventh decade, The decline in plasma renin levels with advancing age has been previously documented in both individuals with normal blood pressure and those with hypertension. In the current study, it was observed that there was a significant increase in plasma norepinephrine levels with advancing age. However, it is important to note that this finding may only partially reflect the expected age-related rise in plasma norepinephrine levels, as previously reported [17]. There was a significant differences between all weight groups when compared control and treated groups as shown in (Fig. 4). This findings is in similarity to the findings of the study [18], which reported that the association between weight and hypertension has been found in most populations, hypertension evaluation clinic screening of more than 1 million persons, the frequency of hypertension in self-reported overweight persons was two to three times higher than that in per-

sons of average and below-average weight individuals, who are overweight or obese experience an increase in vascular resistance due to the presence of excess fat. This, in turn, necessitates the heart to exert additional effort in order to effectively circulate blood throughout the body. Engaging in this additional activity imposes additional cardiovascular burden and leads to elevated blood pressure levels. The present study shows a significant difference between married and single population within control and treated groups (Fig. 5). This result is in consistency with the result of the study [19], research studies have indicated that unmarried males exhibit a greater susceptibility to hypertension compared to their married counterparts. Conversely, unmarried females tend to display a reduced risk of hypertension in comparison to married females.

Consequently, it can be inferred that an individual's marital status is associated with variations in their health status, particularly in relation to hypertension. The precise mechanisms that contribute to the impact of marital status on hypertension remain incompletely comprehended. Prior research has put forth various hypotheses to account for the impact of marital status, encompassing psychopathological factors, neuroendocrine pathways, health behaviors (such as physical activity, diet, and adherence), biological mediators, and immune pathways. There exists a suggestion that married men experience enhanced sleep quality, reduced stress levels, improved mood, and adopt healthier dietary habits in comparison to their never-married counterparts [20, 21]. The current study reported that there was a significant difference between primary, secondary, academic, and uneducated population within control and treated groups. There were non-significant differences between primary level of education when compared control group with treated group as shown in (Fig. 6). This result is similar to that of this study [22]. The findings indicate that individuals who have completed middle school education or higher have a reduced likelihood of developing hypertension and are more successful in managing their blood pressure compared to those with elementary school education or below. Individuals who have completed elementary school education or have a lower level of education may exhibit a higher likelihood of engaging in unhealthy lifestyle behaviors, such as consuming an imbalanced diet or having insufficient physical activity. These behaviors have been linked to the development of hypertension. Furthermore, it has been observed that individuals diagnosed with hypertension who have received an elementary school education or lower may exhibit suboptimal adherence to prescribed antihypertensive medications [23]. The findings of study show a signifi-

cant difference between employee, not employee, and retired within control group (Fig. 7).

The finding of this study was supported by study [24], which states that found a significant association between hypertension awareness and occupational status. The current study show a significant differences between moderate economic status as compared with poor and rich within both control and treated groups, but non-significant differences between poor, rich, and moderate economic status when compared control and treated groups as shown in (Fig. 8). This result is in consistency with the result of the study [25], which shown that lower economic status there is a consistent correlation between this factor and elevated blood pressure levels in the majority of research conducted in developed nations. The strength of this inverse gradient is observed to be greater and more consistently present in women as compared to men. There was a notable decrease in systolic blood pressure among patients who were administered both their own drug and thyme, as opposed to those who received only their own drug. However, this decrease was not statistically significant when compared to the control group. Additionally, there was an observed lack of statistical significance in the reduction of systolic blood pressure among patients who were administered the experimental drug, in comparison to the control group, as depicted in (Fig. 12). The present study's findings were corroborated by a previous study [26], which reported that the administration of rosmarinic acid resulted in a reduction of systolic blood pressure (SBP) specifically in hypertensive rats. This suggests that the observed effects of rosmarinic acid are limited to situations where the renin-angiotensin-aldosterone

system is excessively active, potentially through the inhibition of angiotensin-converting enzyme (ACE). The present study demonstrates a statistically significant reduction in diastolic blood pressure among patients who received a combination of their prescribed medication and thyme, as compared to patients who only received their prescribed medication. However, the decrease in diastolic blood pressure was not found to be statistically significant when compared to the control group. Additionally, there was an observed lack of statistical significance in the reduction of diastolic blood pressure among patients who were administered the experimental drug, in comparison to the control group, as depicted in figure 13.

The present study's findings are consistent with a previous study [27] that demonstrated the efficacy of compounds in reducing blood pressure in both spontaneously hypertensive rats and hypertensive humans. The administration of these compounds resulted in a significant decrease in both systolic and diastolic blood pressure. These promising results suggest that the utilization of an extract derived from *Thymus serpyllum* L. may hold potential for the treatment of hypertension in affected individuals.

CONCLUSIONS

Based on the findings obtained, it can be inferred that thyme herbs have the ability to induce the normalization of hypertension, thereby effectively maintaining blood pressure within the typical range. The available clinical trial data provide evidence for the safe utilization of herbal preparations containing Thyme in the treatment of hypertension.

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PECULIARITIES OF DYNAMICS OF INDICATORS OF PROTEINS OXIDATIVE MODIFICATION AND MATRIX METALLOPROTEINASE-9 ACTIVITY IN PATIENTS WITH PARANOID SCHIZOPHRENIA DEPENDING ON THE DISEASE DURATION

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ABSTRACT

The aim: The objective of the research was to study the indicators of oxidative modification of proteins (OMP) and the activity of matrix metalloproteinase-9 (MMP-9) in patients with paranoid schizophrenia depending on the disease duration.

Materials and methods: 320 patients were included in the examination. 20 patients were with "Primary psychotic episode" (Comparison Group) and 300 patients were diagnosed with "Paranoid schizophrenia" (Experimental Group): 60 of them have suffered from this disease for a duration from 3 to 5 years (Subgroup I); 60 patients have suffered for a period from 6 to 10 years (Subgroup II); 60 individuals – from 11 to 15 years (Subgroup III); 60 patients have suffered for a duration from 16 to 20 years (Subgroup IV); 60 patients – from 21 years and longer (Subgroup V).

Results: The presented data showed that the levels of OMP indicators in Subgroup I constituted 0.826±0.046 conventional units at a wavelength of 356 nm; 0.864±0.051 conventional units at a wavelength of 370 nm; 0.444±0.019 conventional units at a wavelength of 430 nm; 0.176±0.007 conventional units at a wavelength of 530 nm, which is 1.99; 1.6; 1.13 and 1.43 times higher than in the Comparison Group. The content of OMP products was higher by 2.24; 1.74; 1.17, and 1.43 times in Subgroup II, respectively, by 2.4; 1.80; 1.36 and 1.46 times in Subgroup III, respectively; by 2.5; 1.9; 1.4; 1.6 times in Subgroup IV, respectively; by 2.5; 2.02; 1.54; 1.7 times in Subgroup V, respectively. The conducted correlation analysis indicated a direct correlation between OMP indicators and the disease duration. The concentration of MMP-9 in the patients of the Comparison Group was equal to 892.84±87.80 pg/ml, which was 11.2% less compared to the Experimental Subgroup I, where this indicator was 992.84±67.50 pg/ml. MMP-9 constituted 1092.53±47.20 pg/ml on average in the patients of Subgroup II, which was 22.36% higher than in the Comparison Group. This indicator was 1702.84±37.60 pg/ml in Subgroup III, which was 90.7% higher than in the Comparison Group. It constituted 1492.84±47.29 pg/ml in Subgroup IV, which was 67.2% higher than in the Comparison Group; and 2037.21±57.80 pg/ml in Subgroup V, which was more than two times higher than in the Comparison Group (p<0.05). The conducted correlation analysis showed a direct relation between MMP-9 expression and the increase in OMP indicators. This relation was more significant between MMP-9 and OMP products of a neutral nature. The correlation strength between MMP-9 and OMP products of a basic nature was somewhat less significant.

Conclusions: According to the results of the conducted analysis, the examined patients had the signs of decompensation of reactive-adaptive biomolecular mechanisms which activated radical reactions with the subsequent accumulation of oxidation products.

KEY WORDS: oxidative modification of proteins, reactive oxygen species, matrix metalloproteinase-9, paranoid schizophrenia, disease duration, oxidative stress

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INTRODUCTION

According to scientific data, proteins are the main components of most biological systems and make up about 70% of the dry mass of cells and tissues. As a result, it is natural that the proteins sustain the most damage. Certainly, the degree of these damages and especially their biological significance can be very diverse and depend on many factors affecting the body [1, 2]. Therefore, it is not surprising that it is proteins of cell membranes that are primarily exposed to reactive oxygen species (ROS) under the

conditions of oxidative stress (OS) causing cell lysis and depolymerization. It has been scientifically proven that some processes of protein oxidative modification contribute to the loss of protein function, cleavage or aggregation, and some lead to proteotoxicity and disruption of cellular homeostasis [3]. It is important to note that similar modification processes are the initial reaction of the cell to a change in its functioning conditions. It should be noted that products of oxidative modification of proteins (OMP) are quite stable by their nature. As a target for specific neutral

and alkaline proteases action, they are practically not recoverable and, therefore, become a trigger for many pathological processes and OS early marker. In addition, proteins are a natural source of free radicals. In the process of their modification, primary amino acid radicals are formed that can interact with neighboring amino acid residues. Almost all amino acid residues of proteins are capable of oxidation leading to changes in their functions. As a result, proteins begin to lose their natural structure and biological properties, such as metabolic or regenerative ones.

Intensification of the processes of proteins peroxidation depletes antioxidants reserves in the body. In particular, sulfo- and aminohydroxyl groups of amino acids are subject to peroxidation. In relation to the products of protein destruction, they accumulate in the research material in the form of aldehyde- and keto-derivatives of dinitrophenylhydrazones. According to the researchers, oxygen-dependent protein oxidation is an early indicator of damage to organs and tissues, so OMP indicators should be under continuous laboratory control.

More and more available data on schizophrenia pathogenesis focus on neuroinflammatory processes. Lu Y and co-authors report that the expression of matrix metalloproteinases has a close correlation with OS, namely the degree of its manifestation determining the relevance of studying the interaction of the state of the prooxidative-antioxidant system and the expression of matrix metalloproteinases in patients with schizophrenia [4]. In particular, matrix metalloproteinase-9 (MMP-9) cleaves various proteins of the extracellular matrix, significantly contributing to numerous physiological and pathological processes [5]. This extracellular protease is actively involved in the regulation of synaptic plasticity [6]. Its participation in the processes of neurons morphology and their transmission of signals has been scientifically confirmed [7]. Recent studies have confirmed that ROS produced during OS affect the expression of MMP-9, the increase of which increases the risk of cognitive impairment in case of schizophrenia [8]. In addition, according to Dickerson, F et al., people with higher levels of MMP-9 have significantly higher chances of developing schizophrenia [9, 10].

THE AIM

The objective of the research was to study the indicators of oxidative modification of proteins (OMP) and the activity of matrix metalloproteinase-9 in patients with paranoid schizophrenia tracing the peculiarities of their dynamics depending on the disease duration.

MATERIALS AND METHODS

The study was conducted at the premises of Municipal Non-profit Enterprise "Prykarpattia Regional Clinical Center for Mental Health of Ivano-Frankivsk Regional Council" (MNE PRCCMHIFRC) and "Pohonia Psychoneurological Care Home". The study included 320 patients: 20 patients with "Primary psychotic episode" (Comparison Group) and 300 patients with a diagnosis of "Paranoid schizophrenia" (Experimental Group): 60 of them have suffered from this disease for a duration from 3 to 5 years (Subgroup I); 60 patients have suffered for a period from 6 to 10 years (Subgroup II); 60 individuals – from 11 to 15 years (Subgroup III); 60 patients have suffered for a duration from 16 to 20 years (Subgroup IV); 60 patients – from 21 years and longer (Subgroup V). The main criteria for inclusion in the Study Group were as follows: the presence of a "Paranoid schizophrenia" diagnosis and individual consent of the patient. Symptoms of schizophrenia were assessed using the Positive and Negative Syndrome Scale (PANSS). Cases of schizophrenia with the comorbidities of substance-related disorders or mental retardation were excluded. Spectrophotometric analysis of carbonyl groups formed during the interaction of 2,4-dinitrophenylhydrazine (2,4-DNPH) with oxidized amino acid residues of proteins was used to study the intensity of OMP. Determination of the optical density of aldehyde- and ketone-containing dinitrophenylhydrazines was conducted on a spectrophotometer SPECORD M 40 (Germany) of a neutral nature at the wavelength of 356 and 370 nm, and the basic nature at the wavelength of 430 and 530 nm. The content of MMP-9 in the blood plasma of all examined patients was determined by the enzyme immunoassay method by means of "Immuno Chem-2100, Microplate Reader" device using the laboratory kit "The RayBiotech Human MMP-9 Enzyme Immunoassay Kit" (USA) and was expressed in pg/ml. All examinations were conducted after the patients signed the informed consent, the terms of which were approved by the Bioethics Commission of the Ivano-Frankivsk National Medical University. The research was conducted according to the "Rules of Ethical Principles of Conducting Scientific Research with Human Participation" approved by the Declaration of Helsinki (1964-2013) and the ethical and moral and legal norms of the order of the Ministry of Health of Ukraine № 281, November 1, 2000.

Statistical processing of the obtained results was conducted using "STATISTICA 7.0." program (StatSoft, Inc.) and the package of statistical functions of "Microsoft Excel, 2016" program. The reliability of the obtained results was confirmed based on the calculation of the Student's coefficient. Correlation analysis was per-

Table I. The content of the products of proteins oxidative modification in the blood serum of the examined patients

Groups	Products of a neutral nature (M±μ)		Products of a basic nature (M±μ)		
	356 nm., aldehyde derivatives	370 nm., keto derivatives	430 nm., aldehyde derivatives	530 nm., keto derivatives	
Experimental Group, n=300 (conventional units)	Subgroup I, (n=60)	0.826±0.046*	0.864±0.051*	0.444±0.019*	0.176±0.007*
	Subgroup II, (n=60)	0.931±0.045*	0.946±0.067*	0.461±0,024*	0.177±0.005*
	Subgroup III, (n=60)	0.996±0.043*	0.981±0.068*	0.532±0,025*	0.180±0.006*
	Subgroup IV, (n=60)	1.034±0.042*	1.030±0.065*	0.551±0,023*	0.198±0.007*
	Subgroup V, (n=60)	1.048±0.041*	1.100±0.058*	0.606±0,023*	0.209±0.008*
Comparison Group, n=20 (conventional units)		0.414±0.032	0.543±0.031	0.391±0,087	0.123±0.043

Note: * – (p<0.05) the data are reliable between the Comparison subgroup and the studied one

formed using the Spearman method (rank method). Arithmetic mean (M), standard error (±m) was used to describe quantitative characteristics.

RESULTS

The results of the statistical analysis of the obtained data are presented in Table I.

The presented data showed that the levels of OMP indicators in Subgroup I constituted 0.826±0.046 conventional units at a wavelength of 356 nm; 0.864±0.051 conventional units at a wavelength of 370 nm; 0.444±0.019 conventional units at a wavelength of 430 nm; 0.176±0.007 conventional units at a wavelength of 530 nm, which was 1.99; 1.6; 1.13 and 1.43 times higher than in the Comparison Group. The content of OMP products was higher by 2.24; 1.74; 1.17 and 1.43 times in Subgroup II, by 2.4; 1.80; 1.36 and 1.46 times respectively in Subgroup III, by 2.5; 1.9; 1.4; 1.6 times in Subgroup IV, by 2.5; 2.02; 1.54; 1.7 times in Subgroup V.

The comparative characteristic of the optical density ratio of aldehyde- and ketone-containing dinitrophenylhydrazines in the examined patients is shown graphically in Figure 1. Here, the ratio of the values in all studied groups is clearly presented in the form of pie charts.

The dynamics of OMP indicators in the examined patients depending on the main disease duration is presented in Figure 2. According to the presented data, the OMP indicators determined by aldehyde- and keto-derivatives of a neutral and basic nature were significantly higher in the Experimental Group than in the Comparison Group and had an upward trend depending on the disease duration.

The conducted correlation analysis showed that there was a direct correlation between OMP indicators and the disease duration: OPM-356/ duration of the disease was strong (r=+0.82; p<0.05); OMP-370/ disease duration was strong (r=+0.86; p<0.05); OMP-430/duration

of the disease was average (r=+0.44; p<0.05); OMP-530/ duration of the disease was weak (r=+0.17; p<0.05).

The results of the study of MMP-9 level in the serum of the examined patients are shown in Figure 3.

According to the presented data, the concentration of MMP-9 in the patients of the Comparison Group was equal to 892.84±87.80 pg/ml, which was 11.2% less compared to the Experimental Subgroup I, where this indicator constituted 992.84± 67.50 pg/ml. MMP-9 was 1092.53±47.20 pg/ml on average in the patients of Subgroup II, which was 22.36% higher than in the Comparison Group. It constituted 1702.84±37.60 pg/ml in Subgroup III, which was 90.7% higher than in the Comparison Group, and 1492.84±47.29 pg/ml in Subgroup IV, which was 67.2% higher than in the Comparison Group, and 2037.21±57.80 pg/ml in Subgroup V, which was more than two times higher than in the Comparison Group (p<0.05).

The conducted correlation analysis showed a direct relation between the expression of MMP-9 and the growth of OMP indicators. This relation was more significant between MMP-9 and neutral OPM products: OMP-356/MMP-9 – strong (r=+0.78, p<0.05); OMP-370/ MMP-9 – strong (r=+0.71, p<0.05). The strength of the correlation between MMP-9 and OMP products of a basic nature was somewhat less pronounced: OMP-430/ MMP-9 – medium (r=+0.57, p<0.05); OMP-530/MMP-9 – medium (r=+0.43, p<0.05).

DISCUSSION

Today's attention focused on the OMP features and the expression of MMP-9 in patients with schizophrenia, particularly its paranoid form. Unfortunately, as it stands today, there is no single concept of this disease pathogenesis, the underlying complex genetic architecture remains elusive complicating significantly the development of pathogenetic treatment methods, and the mechanisms of its development and progression remain debatable [11].

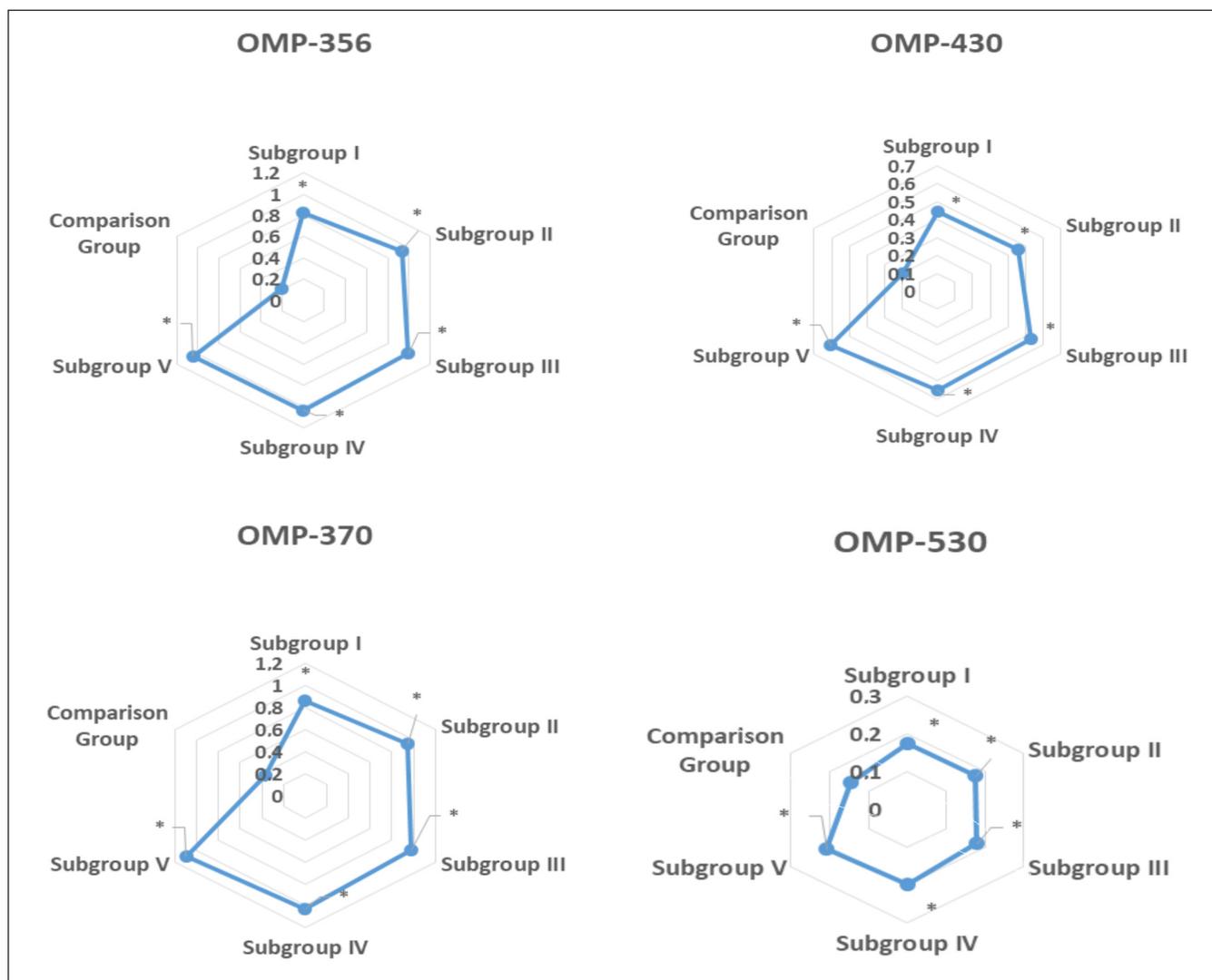


Fig. 1. Ratio of indicators of the optical density of aldehyde- and ketone-containing dinitrophenylhydrazines in the examined patients. Note: * – ($p < 0.05$) the data are reliable between the studied subgroups of Experimental Group and Comparison Group.

Our previous studies established that paranoid schizophrenia was manifested in an imbalance of BDNF and MMP-9 expression that can affect the processes of neurogenesis and synaptic plasticity. According to the PANNS scale, this affects emotions, thinking processes, cognition, causes a loss of interest to social and environmental phenomena, contributes to the deterioration of memory, so they can be considered as diagnostic markers of such pathology [12]. We found that BDNF expression significantly decreased and the expression of MMP-9 increased with an increase in the duration of the studied pathology, and the therapeutic approach of such patients should take these changes into account [13,14].

According to the presented data, the OMP indicators determined by aldehyde- and keto-derivatives of a neutral and basic nature were significantly higher in the Experimental Group than in the Comparison Group and had an upward trend depending on the disease duration. The conducted

correlation analysis showed that there was a direct correlation between OMP indicators and the disease duration

The results of the study of the level of MMP-9 in the serum of the examined patients reflect its tendency to increase depending on the duration of the underlying disease. The conducted correlation analysis showed a direct relation between the expression of MMP-9 and the growth of OMP indicators. This relation was more significant between MMP-9 and neutral OPM products

The complex dynamics of redox regulation mechanisms and their modulation in case of schizophrenia are evidenced by separate scientific works [15]. Currently, there are scarce data that acquaint us with the features of OMP and MMP-9 expression in case of schizophrenia, and there are no data that would reflect the dynamics of this indicator among the specified category of patients, taking into account the duration of their disease. All of the above mentioned confirms the relevance and importance of further research in this direction.

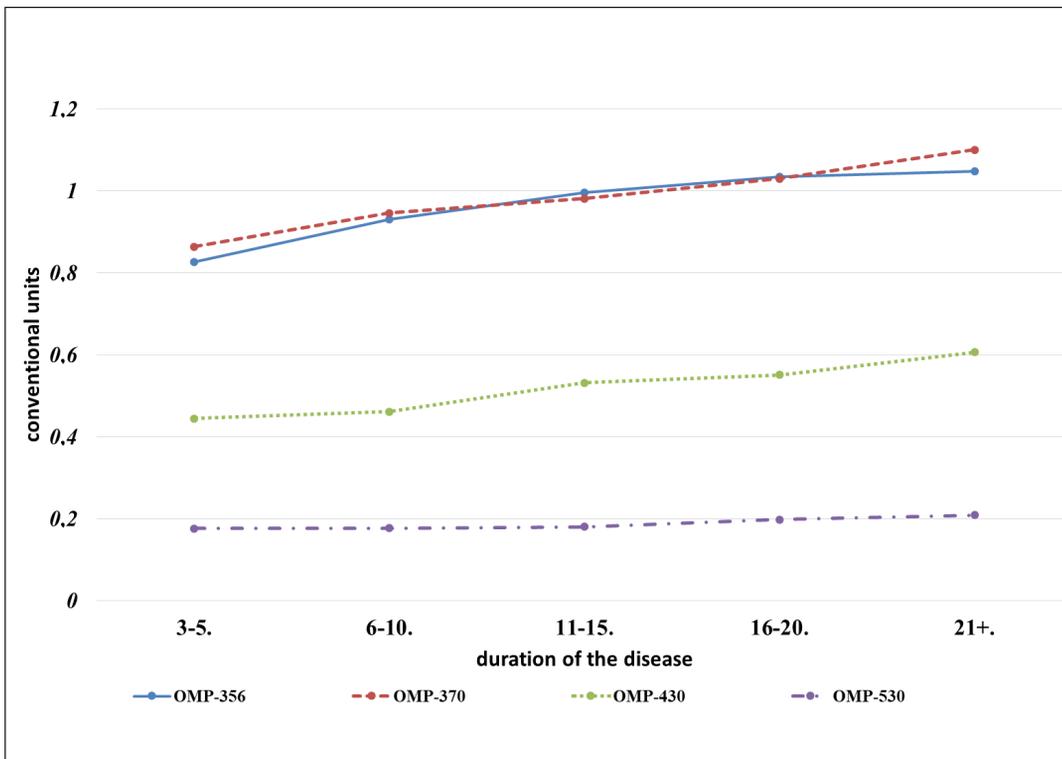


Fig. 2. Dynamics of indicators of oxidative modification of proteins in the examined patients

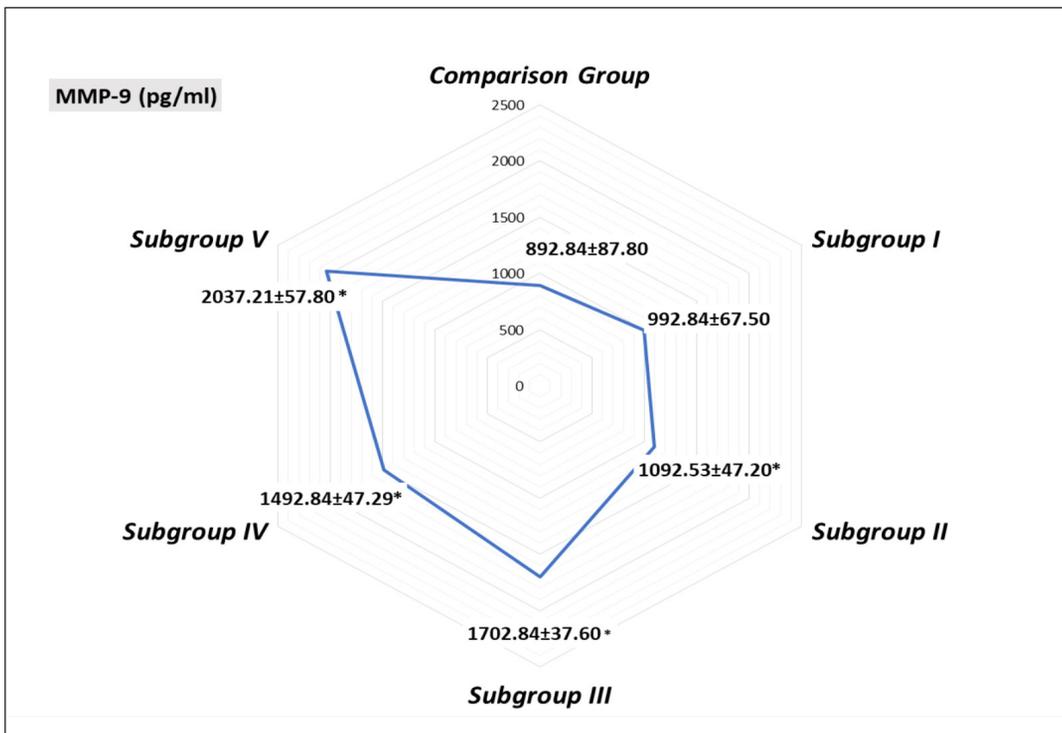


Fig. 3. The level of MMP-9 in the blood serum of the examined patients
Note: * – (p<0.05) the data are reliable between the study subgroups of Experimental Group and Comparison Group.

CONCLUSIONS

Thus, according to the results of the conducted analysis, the examined patients had the signs of decompensation of reactive-adaptive biomolecular mechanisms which activated radical reactions with the subsequent accumulation of oxidation products having a toxic effect. This was evidenced by the accumulation of aldehyde and ketone derivatives in blood serum, which were positively correlated with MMP-9 activity.

Statistical analysis also confirmed a positive correlation between OMP indicators and the duration of the main disease course.

Prospects for further research are to monitor the dynamics of OMP indicators and MMP-9 activity and their mutual influence on the features of pathopsychological symptoms (according to the analysis of the PANSS scale) in the patients with paranoid schizophrenia depending on the duration of their disease.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

EFFECT OF DRY EXTRACT FROM REISHI MUSHROOMS ON THE STATE OF ANTIOXIDANT SYSTEM IN RATS WITH DMH-INDUCED COLON CARCINOGENESIS

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ABSTRACT

The aim: To study pro- and antioxidant systems indicators in rats with chemically induced colon carcinogenesis on the background of the reishi mushrooms dry extract use.

Materials and methods: The study was performed on 120 white male rats. Chronic oncogenic intoxication was modeled by administering 1,2-dimethylhydrazine (DMH) hydrochloride for 30 weeks (1 time per week). A dry extract from the reishi mushrooms was administered intragastrically daily at a dose of 100 mg/kg of the animal's body weight. Blood and liver samples were taken for research monthly. The state of the pro- and antioxidant systems was studied by the content of oxidative modification of proteins products, superoxide dismutase and catalase activity, contents of reduced glutathione and ceruloplasmin.

Results: An increase in the activity of free radical oxidation processes after DMH-induced colon carcinogenesis in rats is evidenced by a decrease in the superoxide dismutase activity, catalase activity, content of reduced glutathione, an increase in the content of ceruloplasmin and products of oxidative modification of proteins in the blood serum and liver of animals. The effectiveness of the dry extract of reishi mushrooms and its positive effect on the state of pro- and antioxidant systems was experimentally proved.

Conclusions: The use of the dry extract of reishi mushrooms under conditions of DMH-induced colon carcinogenesis in rats led to normalization of the antioxidant protection system state and the reduction of oxidative stress.

KEY WORDS: colorectal cancer, dry extract, oxidative stress, reishi mushroom, dimethylhydrazine

Wiad Lek. 2023;76(12):2632-2640

INTRODUCTION

Oncological diseases are one of the main causes of mortality worldwide. It is known about cancer that it is not one disease, but at least 200 – and each of them has its own symptoms, methods of diagnosis and treatment. For many years, cancer has been one of the main components of non-infectious morbidity, disability and mortality of the population in the world, which lead to significant losses of the labor and life potential of society [1, 2]. The onco-epidemiological situation in Ukraine is characterized by high morbidity. Over the past 10 years, the number of cancer patients has increased by 25%. The dynamics of the morbidity and mortality rates of the population of Ukraine over the past 10 years, the characteristics of oncology service, indicate the need to improve all aspects of the anti-cancer fight [3, 4].

The nomenclature of natural drugs with an oncoprotective effect on the pharmaceutical market of Ukraine is insufficient, therefore the search for appropriate raw materials and the creation of new medicinal products based on them is urgent [1, 5, 6].

Reishi mushrooms have a lot of useful properties and are widely used in oriental medicine as drugs that have antibacterial, antiviral, anti-inflammatory, anti-allergenic, antioxidant and anti-tumor effects. Reishi mushrooms are usually used as oncoprotectors together with shiitake and maitake mushrooms. They have been proven to have a beneficial effect on the cardiovascular system, the ability to thin the blood, reduce the blood clotting rate and blood sugar level, expand the coronary vessels of the heart, prevent the development of coronary heart disease, thrombophlebitis and heart attacks, effectiveness in hypertension, arrhythmias, tachycardia, gastritis, ulcers stomach and duodenum, hemorrhoids, diseases of the thyroid gland and liver, respiratory and mental diseases, epilepsy [1, 7, 8]. The advantage of using reishi mushrooms as oncoprotectors is their easy assimilation by the human body, the possibility of long-term use without the risk of side effects, the mildness and reliability of the pharmacological action [9, 10].

THE AIM

The aim of our research was to study pro- and antioxidant systems indicators in rats with chemically induced colon carcinogenesis on the background of the reishi mushrooms dry extract use.

MATERIALS AND METHODS

Experiments were performed on white outbred male rats weighing 190-210 g, which were kept on the standard ration of the vivarium of I. Horbachevsky Ternopil National Medical University. All studies were conducted in compliance with Good Laboratory Practice (GLP) and bioethics in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" [11]. The study was approved by the Ethical Committee of I. Horbachevsky Ternopil National Medical University (Excerpts from the protocol №70, from 01.08.2022).

Experimental study design comprised three groups: 1st – control (C); 2nd – animals affected by 1,2-dimethylhydrazine hydrochloride, control pathology (CP); 3rd – rats affected by 1,2-dimethylhydrazine hydrochloride, treated with a reishi mushrooms dry extract (RMDE). RMDE was administered intragastrically daily at a dose of 100 mg/kg of animal body weight during 30 weeks of the experiment. Dose 100 mg/kg in our previous studies, was found to be conditionally therapeutic for this extract [1]. 1,2-DMH was injected subcutaneously into the interscapular area at a dose of 7.2 mg/kg once a week for 30 weeks, according to the rat's body weight. Animals, which were injected subcutaneously with saline every week, were the control for experimental group of rats. There were 8 animals in the control group, which were euthanized after the first month of the experiment. The 2nd and 3rd groups included 56 animals each. 8 animals from each group were euthanized monthly. [1, 3, 12]

Liver homogenate and blood serum were tested monthly after animal euthanasia. The state of pro-oxidant system in blood serum and animal liver homogenates were evaluated by the content of oxidative modification of proteins (OMP) of neutral and alkaline character. The state of antioxidant system was evaluated by the superoxide dismutase (SOD) and catalase (CAT) activity, by the content of ceruloplasmin (CPL) and reduced glutathione (GSH) [13].

Statistical analysis of the data was performed using STATISTICA 13 (TIBCO Software Inc., 2018). For all indices, the arithmetic mean of the sample (M), lower and upper quartile were calculated. Taking into account the non-normality of the data distribution, non-parametric criteria were used to determine the reliable difference

between independent and dependent indicators. The reliability of the difference between the values between the independent quantitative values was determined by the Mann-Whitney test. The difference between the values was considered probable at $p < 0.05$ [14]. A significant difference between dependent indicators determined by euthanasia of experimental animals at different time points of the experiment was determined on the basis of the Friedman test.

The hypothesis of this study was the assumption of the presence of antioxidant and oncoprotective effects of reishi mushroom extract. This hypothesis is scientific, because it can be denied in the absence of reliable changes in the indicators of antioxidant protection in rats with induced carcinogenesis. To model the latter, the malignant effect of DMH on the colon of experimental animals was used, the effectiveness of which was assessed by the severity of oxidative stress.

RESULTS

The prooxidant-antioxidant status of animals with DMH-induced carcinogenesis was assessed in blood serum and liver homogenate by the content of oxidative modification of proteins products, activity of superoxide dismutase and catalase.

The development of oxidative stress after the introduction of 1,2-DMH is evidenced by the increase in the content of products of oxidative modification of proteins in the blood serum and liver of animals. This can be explained by an excess of free radicals and active forms of oxygen, which were formed due to the negative impact of the toxicant.

When studying the parameters of OMP, it was established that in the blood serum and liver of rats with simulated carcinogenesis, there was a probable ($p < 0.05$) increase in the content of neutral (370 nm) and basic (430 nm) 2,4-dinitrophenylhydrazones (2,4-DNPH), starting from the 2nd month of the experiment (table I, II).

The content of neutral 2,4-DNPH increased by 1.9 times in the blood serum and by 1.5 times in the liver of the affected rats compared to the control on the 3rd month of the study. On the 5th and 7th month (fig 1) of the experiment, an increase of this indicator by 3.3 and 4.0 times was noted in the blood serum and by 2.3 and 2.9 times in the liver of animals with a simulated tumor process.

As can be seen from the table I, the content of 2,4-DNPH of a neutral nature was probably lower both in the serum and in the liver of animals that were injected with 1,2-DMH on the background of the use of RMDE compared to the control pathology.

Table I. Content of neutral 2,4-DNPH ($\mu\text{mol/g}$ protein) in blood serum and liver of rats with DMH-induced colon carcinogenesis, and after the application of reishi mushroom extract ($n=120$).

Period of Affection	Index/Group of animals					
	C		CP		CP+RMDE	
	Serum	Liver	Serum***	Liver***	Serum***	Liver***
1 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,025 0,019; 0,031	0,059 0,051; 0,065	0,024 0,019; 0,028	0,051 0,043; 0,060
2 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,035* 0,030; 0,039	0,065* 0,060; 0,071	0,029 0,024; 0,034	0,062 0,057; 0,067
3 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,043* 0,036; 0,049	0,079* 0,072; 0,085	0,033** 0,031; 0,037	0,068** 0,061; 0,075
4 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,056* 0,050; 0,067	0,102* 0,096; 0,108	0,037** 0,029; 0,047	0,079** 0,073; 0,084
5 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,075* 0,068; 0,079	0,120* 0,105; 0,133	0,041** 0,037; 0,046	0,083** 0,072; 0,093
6 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,083* 0,076; 0,088	0,135* 0,126; 0,147	0,042** 0,037; 0,046	0,095** 0,084; 0,105
7 month	0,023 0,019; 0,029	0,052 0,044; 0,059	0,092* 0,079; 0,103	0,153* 0,141; 0,167	0,044** 0,039; 0,049	0,097** 0,089; 0,106

Note. Here and in the following tables * - probable changes between the index of control and dimethylhydrazine-affected animals, ** - probable changes between the index of dimethylhydrazine-affected and extract-treated animals. *** - probable difference in parameter values in dynamics.

Table II. Content of basic 2,4-DNPH ($\mu\text{mol/g}$ protein) in blood serum and liver of rats with DMH-induced colon carcinogenesis and after application of reishi mushroom extract ($n=120$).

Period of Affection	Index/Group of animals					
	C		CP		CP+RMDE	
	Serum	Liver	Serum***	Liver***	Serum***	Liver***
1 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,018 0,015; 0,021	0,040 0,034; 0,046	0,016 0,014; 0,020	0,041 0,038; 0,046
2 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,023* 0,018; 0,027	0,045* 0,039; 0,051	0,021 0,018; 0,025	0,037** 0,031; 0,041
3 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,033* 0,026; 0,041	0,056* 0,050; 0,062	0,025 0,022; 0,028	0,040** 0,037; 0,044
4 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,047* 0,045; 0,051	0,075* 0,071; 0,081	0,031** 0,025; 0,038	0,045** 0,040; 0,049
5 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,060* 0,056; 0,067	0,094* 0,088; 0,100	0,035** 0,033; 0,039	0,052** 0,046; 0,057
6 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,093* 0,085; 0,100	0,122* 0,119; 0,129	0,041** 0,036; 0,047	0,064** 0,056; 0,076
7 month	0,015 0,013; 0,017	0,036 0,033; 0,041	0,107* 0,098; 0,114	0,144* 0,142; 0,151	0,048** 0,042; 0,055	0,070** 0,061; 0,081

When studying the content of 2,4-DNPH of a basic nature, a probable increase by 1.5 and 1.3 times was noted already on the 2nd month of the experiment in the blood serum and liver of rats with DMD pathology compared to animals of control group. On the 7th month, this indicator increased by 7.1 times in the blood serum and by 4.0 times in the liver of rats with a simulated tumor process compared to the control (fig 1).

Under the influence of RMDE, the content of 2,4-DNPH of the main character was probably lower in the blood serum and liver of rats throughout the experi-

ment in comparison with the group of animals to which no correction was applied.

Changes in antioxidant system of the rat body after the introduction of the toxicant are evidenced by a significant decrease in SOD activity in the blood serum and liver of animals relative to the control, which can be caused by an increase in concentration of hydrogen peroxide and accumulation of compounds that affect the degree of enzyme recovery (table III).

The introduction of a dry extract from reishi mushrooms into the affected body led to a probable increase of SOD

Table III. Superoxide dismutase activity in blood serum and liver (mcat/g protein) of rats with DMH-induced colon carcinogenesis and after the use of reishi mushroom extract (n=120).

Period of Affection	Index/Group of animals					
	C		CP		CP+RMDE	
	Serum	Liver	Serum***	Liver***	Serum***	Liver***
1 month	60,81 55,94; 65,44	45,96 42,77; 49,18	58,72 55,81; 61,66	47,46 43,68; 50,94	59,06 55,50; 63,81	44,87 41,21; 48,80
2 month	60,81 55,94; 65,44	45,96 42,77; 49,18	56,47 52,90; 59,13	45,04 42,73; 49,10	59,11 57,41; 60,48	43,75 40,16; 47,04
3 month	60,81 55,94; 65,44	45,96 42,77; 49,18	53,06* 49,10; 56,00	40,03* 38,08; 42,30	58,23** 56,08; 58,86	42,51 40,51; 44,46
4 month	60,81 55,94; 65,44	45,96 42,77; 49,18	47,46* 44,19; 51,87	35,75* 32,47; 38,73	55,22** 54,12; 56,55	40,19 38,85; 42,85
5 month	60,81 55,94; 65,44	45,96 42,77; 49,18	41,66* 36,90; 46,38	32,56* 30,92; 35,08	53,84** 51,31; 55,72	38,90** 38,08; 40,95
6 month	60,81 55,94; 65,44	45,96 42,77; 49,18	38,22* 33,21; 41,87	27,99* 24,86; 32,13	50,01** 48,34; 53,20	37,88** 34,74; 40,48
7 month	60,81 55,94; 65,44	45,96 42,77; 49,18	31,86* 28,30; 36,83	24,56* 20,00; 28,97	48,47** 45,89; 49,82	36,65** 34,09; 38,65

Table IV. Catalase activity in blood serum (mcat/l) and liver (mcat/kg) of rats with DMH-induced colon carcinogenesis and after application of reishi mushroom extract (n=120).

Period of Affection	Index/Group of animals					
	C		CP		CP+RMDE	
	Serum	Liver	Serum***	Liver***	Serum***	Liver***
1 month	3,79 3,47; 4,10	5,93 5,50; 6,32	3,46 3,21; 3,80	5,87 5,53; 6,18	3,95** 3,81; 4,22	6,04 5,88; 6,25
2 month	3,79 3,47; 4,10	5,93 5,50; 6,32	3,22 2,78; 3,67	5,66 5,36; 5,90	3,72 3,48; 3,97	5,88 5,66; 6,00
3 month	3,79 3,47; 4,10	5,93 5,50; 6,32	3,07* 3,01; 3,21	4,75* 4,36; 5,09	3,64** 3,47; 3,91	5,46** 5,10; 5,70
4 month	3,79 3,47; 4,10	5,93 5,50; 6,32	2,68* 2,39; 2,97	4,07* 3,87; 4,30	3,44** 3,30; 3,67	5,29** 5,11; 5,64
5 month	3,79 3,47; 4,10	5,93 5,50; 6,32	2,23* 1,97; 2,49	3,80* 3,58; 4,09	3,29** 3,23; 3,53	5,12** 4,85; 5,39
6 month	3,79 3,47; 4,10	5,93 5,50; 6,32	1,99* 1,80; 2,21	3,15* 3,02; 3,38	3,21** 3,00; 3,44	4,99** 4,78; 5,23
7 month	3,79 3,47; 4,10	5,93 5,50; 6,32	1,74* 1,50; 1,87	2,77* 2,52; 3,07	2,98** 2,80; 3,16	4,69** 4,58; 4,86

activity in the blood serum already on the 3rd month of the experiment by 1.1 times compared to the control pathology. When investigating the effect of RMDE on SOD activity in the liver of animals with DMH-induced carcinogenesis, it was established that a probable increase in the activity of the enzyme by 1.2 times relative to the affected rats occurred on the 5th month of the experiment.

White rats' affection with a carcinogen caused a decrease in catalase activity in the blood serum and liver of animals (table IV). Due to insufficient CAT activity, hydrogen peroxide accumulates, which leads to the appearance of lipid hydroperoxides and oxidative modification of proteins.

The activity of the enzyme in blood serum decreased by 19%, in the 5th month this indicator decreased by 41%, in the 7th month – by 54% compared to the control on the 3rd month after the injury. Catalase activity in the liver of rats under the influence of DMH decreased by 20%, 36%, and 53% on the 3rd, 5th, and 7th months of the study, respectively, relative to animals of control group (figure 2).

A positive trend was observed after the correction with RMDE with regard to catalase activity, which already was probably by 1.2 times higher in blood serum and by 1.1 times higher in the liver of animals compared to the control pathology on the 3rd month of the experiment (table IV).

Table V. Content of CPL (mg/l) in the blood serum of rats with DMH-induced colon carcinogenesis and after application of reishi mushroom extract (n=120).

Period of Affection	Index/Group of animals		
	C	CP***	CP+RMDE ***
1 month	3,46 [3,22; 3,80]	3,35 [3,12; 3,64]	3,63 [3,19; 3,80]
2 month	3,46 [3,22; 3,80]	4,10 [3,18; 5,24]	3,79 [3,36; 4,03]
3 month	3,46 [3,22; 3,80]	4,59* [3,73; 5,24]	3,95 [3,42; 4,40]
4 month	3,46 [3,22; 3,80]	5,50* [5,28; 5,71]	4,18** [3,85; 4,57]
5 month	3,46 [3,22; 3,80]	6,04* [5,65; 6,44]	4,32** [3,97; 4,92]
6 month	3,46 [3,22; 3,80]	7,14* [6,69; 7,69]	4,70** [4,25; 5,07]
7 month	3,46 [3,22; 3,80]	7,90* [7,56; 8,25]	4,80** [4,57; 4,98]

Table VI. GSH content (mmol/g protein) in serum and liver of rats with DMH-induced colon carcinogenesis and after application of reishi mushroom extract (n=120).

Period of Affection	Index/Group of animals					
	C		CP		CP+RMDE	
	Serum	Liver	Serum***	Liver***	Serum***	Liver ***
1 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	1,30 [1,21; 1,39]	2,01 [1,88; 2,19]	1,37 [1,26; 1,46]	1,96 [1,87; 2,06]
2 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	1,20* [1,13; 1,27]	1,89 [1,79; 2,05]	1,31 [1,26; 1,39]	1,90 [1,81; 2,00]
3 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	1,06* [0,98; 1,15]	1,61* [1,43; 1,75]	1,24** [1,17; 1,29]	1,86** [1,80; 1,94]
4 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	0,91* [0,83; 1,02]	1,42* [1,36; 1,46]	1,17** [1,09; 1,24]	1,79** [1,69; 1,88]
5 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	0,74* [0,64; 0,82]	1,28* [1,19; 1,35]	1,09** [0,99; 1,18]	1,72** [1,58; 1,87]
6 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	0,60* [0,52; 0,67]	1,09* [0,93; 1,27]	1,03** [0,91; 1,09]	1,67** [1,59; 1,76]
7 month	1,33 [1,28; 1,39]	1,94 [1,89; 2,01]	0,52* [0,44; 0,61]	0,95* [0,84; 1,10]	0,97** [0,89; 1,08]	1,57** [1,45; 1,71]

It was noted a probable increase in the activity of the enzyme by 1.8 times in blood serum and by 1.7 times in the liver of rats relative to animals with DMH-induced carcinogenesis on the 7th month of the research.

The next stage of our research was to study the content of CPL in the blood serum of rats affected by DMH and after correction with RMDE (fig 3). CPL has pronounced oxidative activity, it limits the release of iron reserves, activates the oxidation of ascorbic acid, norepinephrine, serotonin and sulfhydryl compounds, inactivates reactive oxygen species, preventing the peroxidation of lipids in the cell membrane, can modulate the function of endothelial nitric oxide synthase, regulating NO-dependent relaxation vessels.

As can be seen from the data in table V, the content of CPL in the blood serum of animals after exposure to a toxic agent increased significantly ($p \leq 0.05$) starting from the 3rd month of the experiment compared to the group of control animals. The use of RMDE in a dose of 100 mg/kg of animals body weight showed a positive effect on the content of this enzyme, probably reducing it by the 4th month of the study.

Reduced glutathione (GSH) belongs to endogenous water-soluble antioxidants, is a cofactor and substrate of the enzymatic antioxidant system, and has a direct neutralizing effect on free radicals.

A probable ($p \leq 0.05$) decrease in the content of GSH in the blood serum of rats was noted from the 2nd month of the experiment by 1.1 times, respectively,

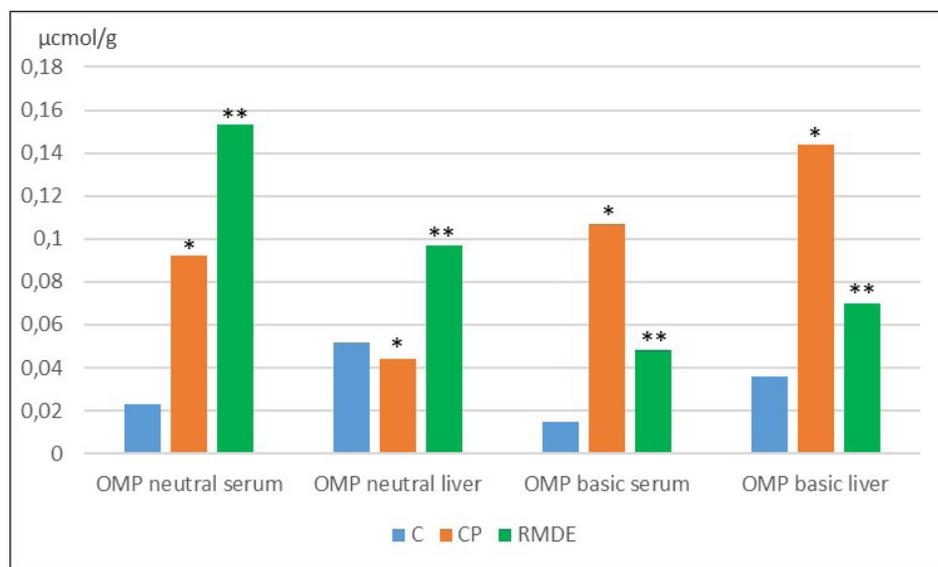


Fig. 1. The content of neutral and basic 2,4-DNPH in the blood serum and liver of rats with DMH-induced colon carcinogenesis and after the application of reishi mushroom extract, 7th month

Note. Here and in the following figures * - probable changes between the index of control and dimethylhydrazine-affected animals, ** - probable changes between the index of dimethylhydrazine-affected and extract-treated animals

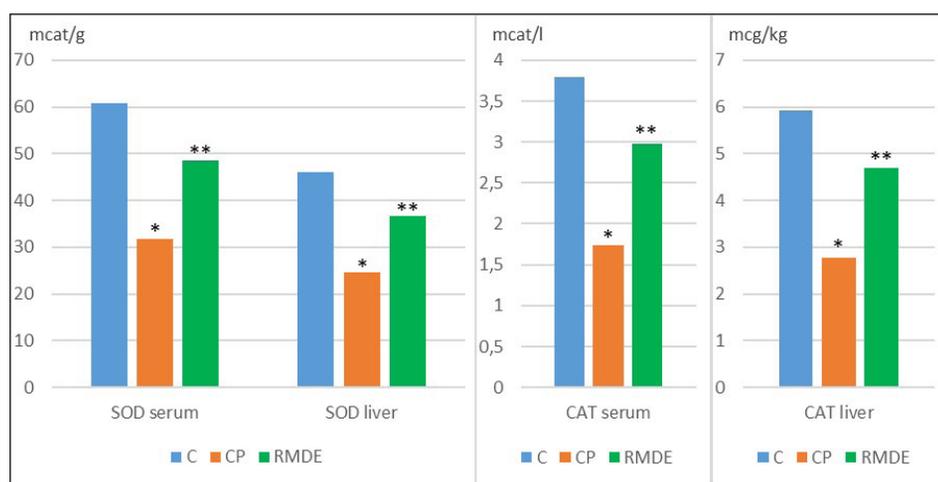


Fig. 2. SOD and CAT activity in serum and liver of rats with DMH-induced colon carcinogenesis and after application of reishi mushroom extract, 7th month.

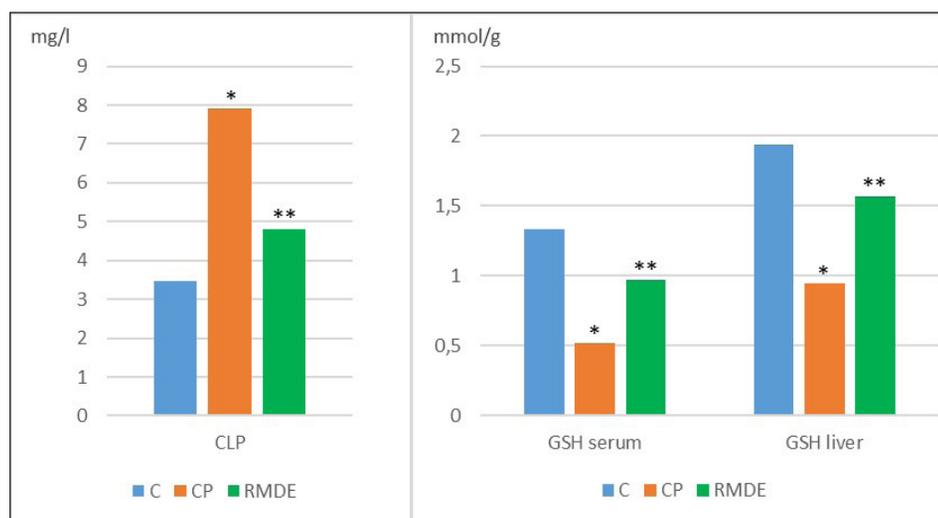


Fig. 3. Content of CPL in the blood serum and content of GSH in the blood serum and liver of rats with DMH-induced colon carcinogenesis and after the application of reishi mushroom extract, 7th month.

compared to the control group (table VI). By the end of the experiment, this indicator decreased by 2.6 times relative to C. A similar tendency was observed in the liver of animals with DMH pathology. Administration of SEGR to animals under the conditions of DMH-induced carcinogenesis led to a probable increase in the content of GSH in the blood serum and liver of rats as early as the

3rd month of the experiment. By the end of the study, the content of GSH in the blood serum and liver of the affected rats, which were injected with the studied extract, increased by 87% and 65%, respectively, relative to the control pathology.

To confirm the detected changes in biochemical indicators and the development of colorectal cancer in

animals, we conducted a histopathological study. The development of DMH-induced colon carcinogenesis in white rats was confirmed histologically. The results of this research are covered in scientific publications [5, 7].

DISCUSSION

The process of oncopathology development is accompanied by a change in the redox balance and disturbances in the antioxidant defense system. The study of the state of the pro- and antioxidant system in the model of DMH-induced carcinogenesis in rats showed a probable increase in the content of OMP products in the blood serum and liver of animals relative to the control. This indicates the development of a pathological process in the affected body. Oxygen-dependent oxidation of proteins is an early indicator of damage to organs and tissues, and the processes of oxidative modification of proteins in all pathological conditions must be under continuous laboratory control [15-18].

Catalase and superoxide dismutase are the main components of the enzyme link of the body's antioxidant defense. SOD regulates the conversion of highly reactive superoxide anion into less active hydrogen peroxide. Catalase, in turn, inactivates the product of SOD reaction with water and molecular oxygen. As a result of the work of these enzymes, the formation of the hydroxyl radical, which is the most active oxidant, is sharply reduced [15, 19].

In the conditions of DMH-induced carcinogenesis, the activity of SOD, which is a key enzyme of antiradical protection, and CAT, which has a significant effect on the intracellular concentration of reduced glutathione and plays a decisive role in neutralizing free radicals, was investigated [12].

It has been experimentally proven that long-term administration of 1,2-DMH leads to an imbalance in the functioning of the antioxidant defense system of the blood and liver of rats, which is manifested by a decrease in SOD and CAT activities, an increase in the content of CP and a decrease in the content of GSH. A decrease in catalase and superoxide dismutase activities in blood serum and liver of experimental rats may indicate 1,2-DMH induction of oxidative stress. This is also indicated by a change in the content of GSH and CP in the blood serum and liver of affected animals, which are one of the markers of the intensity of lipid peroxidation and the development of oxidative stress [19].

Analyzing the dynamics of changes in the activity of the studied enzymes and the content of OMP products in the blood serum and liver homogenate of affected rats, which were injected with RMDE for 7 months, it was established that there was a probable increase in the activity of antioxidant protection enzymes and a

decrease in the content of OMP products in all studied tissues of the affected animals. Thus, the effective influence of RMDE on the pro-oxidant-antioxidant state of the liver and blood of rats with a simulated oncological process was proven [20, 21].

In the future, it is planned to study the effect of reishi mushroom extract on the activity of glutathione peroxidase and glutathione reductase in DMH-induced colon carcinogenesis. The antioxidant effect of reishi will be investigated on models of other pathologies, which are accompanied by the activation of oxidative processes.

CONCLUSIONS

The obtained results of the study allow us to state that the lesion of rats with 1,2-DMH leads to the activation of free radical oxidation processes and a compensatory decrease in the activity of the studied enzymes with the subsequent depression of antioxidant protection. This is evidenced by an increase in the content of 2,4-DNPH and CPL, a decrease in SOD and catalase activity, a decrease in the content of GSH in the blood serum and liver of animals with a simulated tumor process. Thus, by the end of the experiment, the following changes were observed after the introduction of 1,2-DMH: the content of neutral and basic 2,4-DNPH probably increased by 4.0 and 7.1 times, respectively, in blood serum and by 2.9 and 4.0 once in the liver of affected animals; the activity of SOD and CAT decreased by 1.9 and 2.2 times in blood serum and by 1.9 and 2.1 times in the liver of animals with DMD pathology; the content of CP and reduced glutathione in blood serum decreased by 2.3 and 2.6 times compared to the control group. This indicates the feasibility of DMH using to model oncogenesis in rats.

The introduction of RMDE for the correction of simulated pathology showed an effective positive influence on the normalization of the studied indicators, caused a gradual stimulation of the antioxidant defense system and a decrease in the content of OMP products in the body during experimental carcinogenesis. Thus, on the 7th month of the study, the content of neutral and basic 2,4-DNPH probably decreased under the influence of reishi extract by 2.1 and 2.2 times, respectively, in blood serum and by 1.6 and 2.1 times in the liver of animals. The activity of SOD and CAT increased by 1.5- and 1.7-fold, respectively, in the serum and liver of rats treated with RMDE, relative to affected animals. This is probably a consequence of the antioxidant and oncoprotective effect of the studied pharmacological drug, which can be a reasonable basis for further study of RMDE. The revealed probable positive dynamics of antioxidant protection indicators allows to confirm the assumption about the effectiveness of reishi extract.

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SAMPLE SIZE CALCULATION IN STRUCTURAL EQUATION MODELING OF EQUILIBRIUM

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ABSTRACT

The aim: To pave the way and exemplify sample size calculation to studies with complex data structures describing equilibrium.

Materials and methods: Try is probated to apply ad-hoc power analysis to structural equation modeling (SEM) of equilibrium. As example we use theoretical structural equation system that describes equilibrium of price, quality and comfort of health services developed by Dranove D., Satterthwaite M. (1992). We show the way of transition from theoretical balance models to SEM that can be processed with common statistical tools. SEM is prerogative for such transition as demonstrated in the paper. We introduced some new ideas to support transition. We use Satorra & Sarris (1985) method of ad-hoc power analysis to SEM.

Results: The sample size to support error types 1 and 2 at arbitrary accepted levels 0.05 and 0.2 is 400 at least to test the influence of equilibrium price (p^*) on equilibrium quality (q^*). 600 sample size is needed to check for the influence of equilibrium price (p^*) on equilibrium comfort (c^*). Sample size of 600 is required to test hypothesis on informational noise about quality influences equilibrium value of quality.

Conclusions: It's new ground that we are tentatively exploring in paper concerning SEM of equilibrium. The main challenge as we see it is the transition from theoretical balance models to SEM that can be processed with common statistical tools. SEM is prerogative for such transition as demonstrated in the paper.

KEY WORDS: SEM, equilibrium, Power Analysis, Health Services Efficiency

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INTRODUCTION

Sample size calculus is important to put a credit to p-value of effect. Take for example historical Tromboembolism Data. This case-control data first considered by Worcester J. (1971) [1]. The data cross-classify thromboembolism and control patients by two risk factors: oral contraceptive user and smoking. Data are regularly used to compare count data estimators, and in subsequent model choice studies, such as Spiegelhalter and Smith (1982) [2], Pettit and Young (1990) [3], Congdon P. (2005) [4], Ocheredko O (2019) [5]. Under the potentially informative priors used, the Bayes factor estimate was $B_{21} = 23.8$, quite strongly in favour of the smaller model with single interaction effect contraceptive*thromboembolism which in all tested estimators proved to be significant. The question is should we put a credit to these findings given the original sample size of 174? To resolve the issue we have to do power analysis. This example is simple to get on but what if we have complex data structure with some variables unobservable or measured with error? What if records are structurally related (e. g., evince nesting, spatial or temporal correlation patterns)? The pick of the bunch is structural equation modeling (SEM) that usually

applied to complex data that support simultaneous or consequential testing of multiple hypotheses. Particular difficulties are imposed by modeling equilibrium processes. Challenges arise at the stage of transition from theoretical structural equations that define equilibrium process (momentum or dynamic) to operational SEM formulation.

There are two general branches of power analysis, ad-hoc and post-hoc. Ad-hoc power analysis uses expert opinion on parameters comprised by statistical test, for example expected difference of two indexes and its sample error for 2 independent samples t-test. Post-hoc counterpart relies both on expert opinion and preliminary data. Actually data can be fed from accomplished study to check the relevancy of p-values.

Structural equations sometimes are substantiated by theoretical equations, which is the common case in health econometric applications. The obstacle to overcome is that theoretical equations may include unobservable variables, like survival skills, patient's utilities, satisfaction, et al. In our test case theoretical econometric equations of Dranove D., Satterthwaite M. include demand elasticities, as well as equilibrium values that are either unknown or unobservable or both.

It's very important to transform such theoretical equations into practical structure supported by statistical data. That's why we cover such transformation briefly, following with sample size calculation on resultant structural equation model.

Overall this paper focuses on power/ sample size issue that is important to support complex data describing equilibrium and in that it blazes the trail.

THE AIM

The aim of the study was to pave the way and exemplify sample size calculation to studies with complex data structures describing equilibrium.

MATERIALS AND METHODS

These includes explaining of (i) theoretical structural equations used in example, (ii) conversion of theoretical structural equations to structural equation model, (iii) power analysis technique.

Theoretical structural equations used in example are derived based on profit maximizing behavior of provider who seeks for profit maximizing levels of three attributes: price (p), quality of services (ql), and comfort (cm). Demand is function of these, i.e., q(p,ql,cm), decreasing in price and increasing in ql and cm. The total cost are function of demand q, as well as ql and cm, so that C(q, ql, cm)=q•(a+b•ql+c•cm) + F with a+b•ql+c•cm is constant marginal cost of production and F is fixed costs. So, the profit is the function of p, ql, and cm:

$$\text{Profit} = p \cdot q \cdot (p, ql, cm) - C(q, ql, cm), \quad q, ql, cm) = q(p, ql, cm)(p - b \cdot ql - c \cdot cm) - F$$

By taking first derivatives by attributes p, ql, cm and solving for their optimal (equilibrium) values given attributes elasticities of demand ($\eta_p^q, \eta_{ql}^q, \eta_{cm}^q$) we can conceptualize theoretical model in system of three simultaneous equations derived by Dranove D., Satterthwaite M. (1992) [6]:

$$p^* = \frac{a\eta_p^q}{1 + \eta_p^q + \eta_{ql}^q + \eta_{cm}^q} = \frac{(a + b \cdot ql^* + c \cdot cm^*)\eta_p^q}{1 + \eta_p^q}$$

$$ql^* = \frac{a\eta_{ql}^q}{b(1 + \eta_p^q + \eta_{ql}^q + \eta_{cm}^q)} = \frac{p^* \eta_{ql}^q}{b \eta_p^q}$$

$$cm^* = \frac{a\eta_{cm}^q}{c(1 + \eta_p^q + \eta_{ql}^q + \eta_{cm}^q)} = \frac{p^* \eta_{cm}^q}{c \eta_p^q}$$

Theoretical equations can't directly be put to practicalities (e.g., statistical regression or power analyses) because they include elasticities of demand ($\eta_p^q, \eta_{ql}^q, \eta_{cm}^q$) that are unavailable ad hoc as well as equilibrium values of price, (p*), quality (ql*), and comfort (cm*) of services which are not directly available and that can only be elucidated by appropriately built model frame.

CONVERSION OF THEORETICAL STRUCTURAL EQUATIONS TO THE STRUCTURAL EQUATION MODEL

First of all we defined non-measurable variables with the related observables. In terms of SEM non-measurable variables are latent factors. Observables are measurable variables used to define latent factors by loadings. Latter are essentially regression coefficients so that latent factor regressed on related observables and so defined by them. For instance, quality of hospital services (F2) is latent factor defined by hospital length of stay, surgery complications risks, quality of personnel, etc. The other latent factors are comfort of hospital services (F3), and information noise (F1). Presence of F1 is a trick to render elasticities effects for F1 greatly influences all three of them ($\eta_p^q, \eta_{ql}^q, \eta_{cm}^q$). There are no demand elasticity coefficients per se among associations. Their influence on equilibrium values of attributes is traceable through associations of F1 with price, F2, and F3. Given their key role in equilibrium related hypotheses formulation correspondent arrows rendered in red in Fig.1. Equilibrium values are substituted with observed values of attributes. It is sensible for theoretical demand and realized demand for surgeries almost coincide. Would we had have arrived at significant regression effects of F1 on price (b11 in Fig.1), quality of hospital services (z1), and comfort (z2) we would conclude that observed values of attributes are not so far from the equilibrium and so we have efficient production of health services. Supportive to theoretical equilibrium equations of Dranove D., Satterthwaite M. are regression effects of price on quality of hospital services (b31) and comfort (b31) that are colored green.

We use standard graph presentation of SEM (Fig.1). Latent factors are encircled and named with beginning letter "F", observable variables are beveled with rectangles, single-headed arrows denote directional associations, while two-headed indicate variances and covariative associations. Names of factor loadings in graphical presentation usually start with "f". Numbers assigned to arrows infer the magnitude and direction of associations. Graphical SEM model as depicted in Fig.1 is produced in special SEM tools environment accessible through <https://webpower.psychstat.org/wiki/> with details given by authors [7].

Values of model's parameters (i.e., variances, covariance, regression coefficients, factor loadings) are retrieved from published sources. These and practicalities of conversion of given theoretical structural equations to the structural equation model is delivered at length in [8].

POWER ANALYSIS TECHNIQUE

Having defined SEM in graphical form like that described by Fig.1 is enough to proceed to power calculus. Given

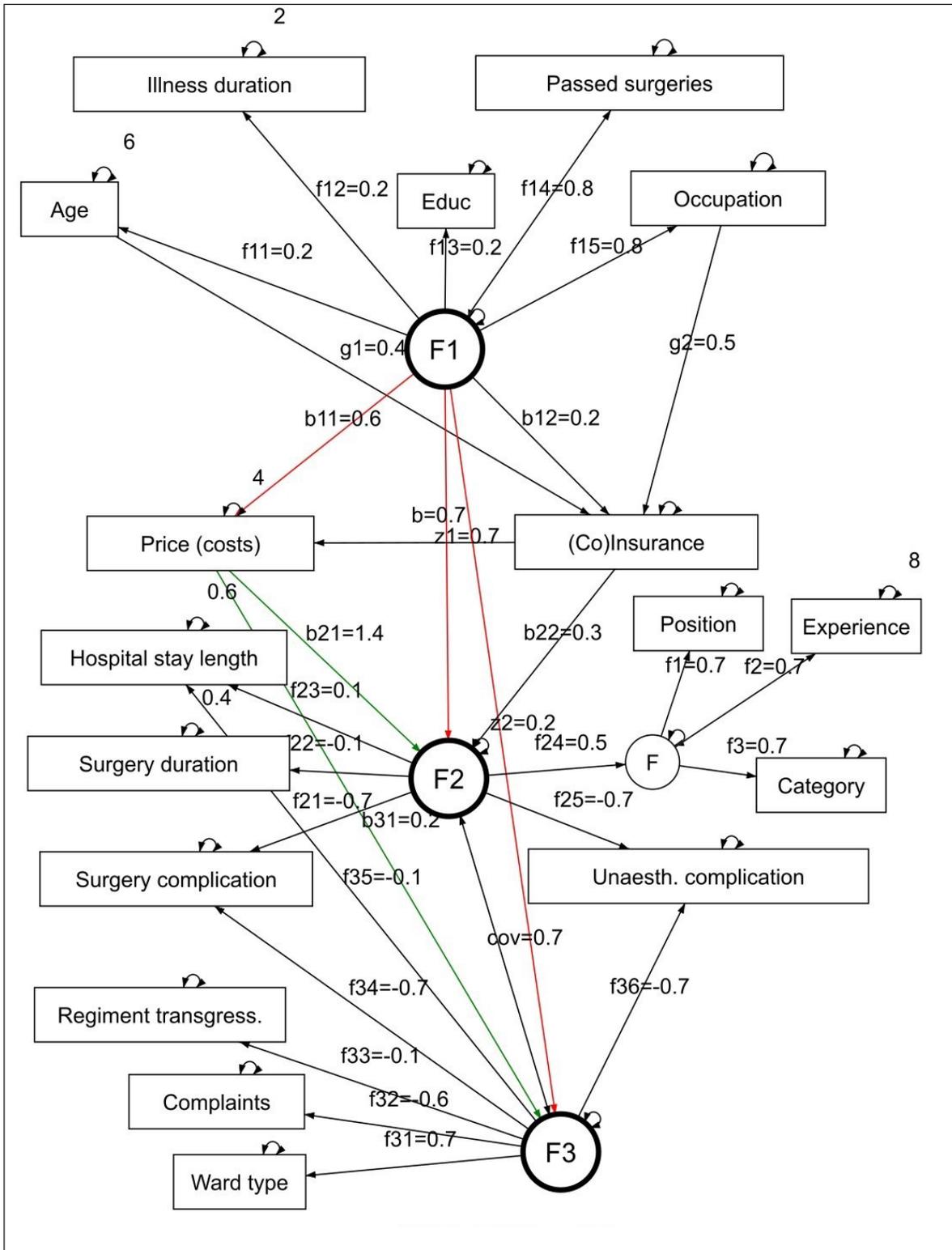


Fig.1. SEM model graph

SEM complexity and possible parameters dependency introduced by latent factors power routine should be based on statistical test that incorporate covariance matrix of parameters or technically speaking Hessian. These should be produced by routine. The commonly used under circumstances is likelihood ratio test. The pick of the bunch is the

one based on chi-square test implemented with Satorra & Sarris (1985) method [9]. In brief let S denote an unbiased sample covariance matrix and θ let denote parameters in a SEM model. Let Σ be the covariance matrix defined by the model with parameters θ . From SEM theory, statistic $W = (n-1) \log |\hat{\Sigma}(\theta)| + \text{tr}(S \hat{\Sigma}(\theta)^{-1}) - \log |S| - p$

```

full.model <- '
f1 =~ start(0.2)*age + start(0.2)*t_ill +start(0.8)*srg +
start(0.2)*educ +start(0.8)*occup
age =~ start(6)*age
t_ill =~start(2)*t_ill
cost =~start(4)*cost

cost ~ start(0.6)*f1 + start(0.7)*insur
insur ~ start(0.2)*f1 +start(0.4)*age +start(0.5)*occup

t_stay =~ start(0.6)*t_stay
t_surg =~ start(0.4)*t_surg
c_surg =~ start(1.0)*c_surg

f2 =~ start(0.1)*t_stay + start(-0.1)*t_surg +start(-0.7)*c_surg +
start(-0.7)*c_anesth + start(0.5)*f0
f2 ~ start(0.3)*insur + start(1.4)*cost + start(0.7)*f1

expert =~ start(8)*expert
f0 =~ start(0.7)*expert + start(0.7)*pos + start(0.7)*cat
f3 =~ start(-0.1)*t_stay + start(-0.1)*t_surg +start(-0.7)*c_surg +
start(-0.7)*c_anesth + start(-0.1)*defect + start(-0.6)*compl + start(0.7)*ward
f3 ~ start(0.2)*cost + start(0.2)*f1
f2 =~ start(0.7)*f3
'
    
```

Fig. 2. Model description in SEM language (R)

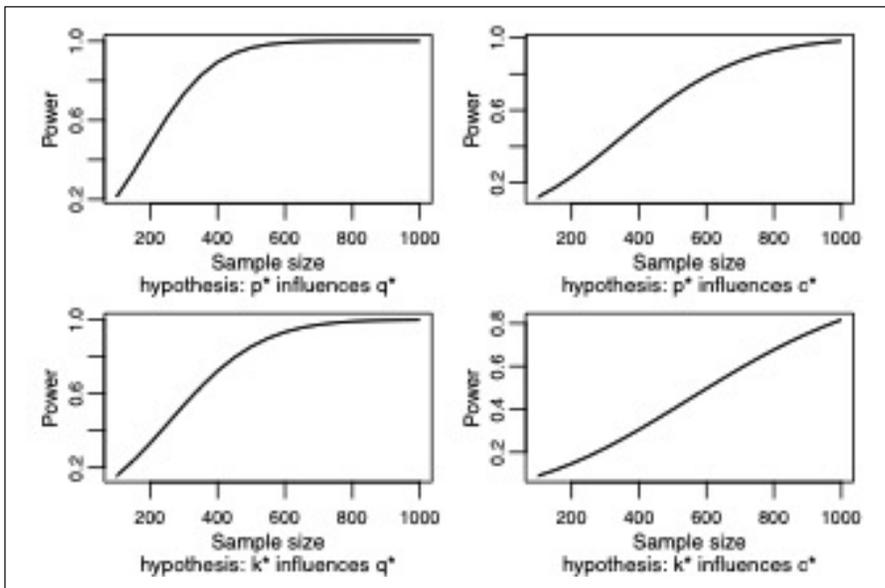


Fig.3. Power curves to test 4 hypotheses: equilibrium price (p*) influences equilibrium quality (q*) and comfort (c*), informational noise about quality and comfort influence q* and c* respectively

follows a chi-squared distribution with degrees of freedom d asymptotically. The purpose is to test the hypothesis that $H_0: \theta = \theta_0$ vs $H_1: \theta = \theta_1$. Under H_0 , we have $P(\chi^2_d > c_\alpha) = \alpha$ where c_α is the critical value under the chi-squared distribution with degrees of freedom d . Under H_1 , W follows asymptotically a non-central chi-squared distribution with the non-centrality parameter λ . The statistical power is defined as $\text{Power} = P(W > c_\alpha | H_1)$.

Satorra & Sarris (1985) showed that λ can be approximated by

$$\lambda \approx (n - 1)[\log |\hat{\Sigma}_R| + \text{tr}(\hat{\Sigma}_F \hat{\Sigma}^{-1}) - \log |\hat{\Sigma}_F| - p]$$

where $\hat{\Sigma}_F$ and $\hat{\Sigma}_R$ are defined under H_1 and H_0 , respectively. With this, one can define an effect size independent of sample size as $\delta = \lambda / (n - 1)$. The effect size is defined as the difference between two SEM models, a full model M_F and a reduced model M_R . The full model

(correct population model) includes all the parameters in the population (Fig.1) and the reduced model is nested within the full model by setting certain relationship to be null (H_0 hypothesis). An easy way to get the effect size is to fit the reduced model to Σ_F through SEM software with a predefined sample size n_F to get the chi-squared statistics λ . In depth technicalities of the approach are given in Yuan K.-H., Zhang Z., Zhao Y. [10]

We have checked four hypotheses, each H_0 hypothesis is described in SEM language first two concern price influence quality (F2) and comfort (F3). For these H_0 hypotheses are represented by removal from full model arrows defined coefficients b21 and b31 that formulates reduced models 1 and 2 (Fig. 1). Third and fourth hypotheses check for informational noise consequence on F2 and F3, so that H_0 hypotheses are represented by

removal from full model arrows defined coefficients z_1 and z_2 (reduced models 3 and 4).

We used R package *WebPower* to do calculus. Function *sem()* is used to calculate covariance matrix, *sem.effect.size()* produces effect size, *wp.sem.chisq()* works out power reckoning, *plot()* method for class 'webpower' is used to plot the power curves.

RESULTS

We don't show covariance matrix for lack of space but calculus is reproducible given model description of full model (Fig. 2)

Code is standard for SEM and having Fig.1 is easy to read for it describes the graphical model. Values in parentheses following *start* keyword are those displayed by graph. Effect sizes estimates play key role in power analysis. They are evaluated by *sem.effect.size()* functions with two arguments which are full model, obtained by code of Fig.2 and reduced model obtained with the same code but with tested parameters put with zeroes, i.e., *start(0)*parameter_name*.

So, we retrieve effect sizes for four hypotheses by code:

```
effect.res1 <- sem.effect.size(full.model, reduced.model1)
effect.res2 <- sem.effect.size(full.model, reduced.model2)
effect.res3 <- sem.effect.size(full.model, reduced.model3)
effect.res4 <- sem.effect.size(full.model, reduced.model4)
```

Finally, we build power curve objects based on effect sizes with the help of function *wp.sem.chisq()* with arguments indicating range of sample sizes to study (n), degrees of freedom of the chi-squared test (df) and effect size (effect) both calculated by *sem.effect.size()* functions.

```
pwr.curve1 <- wp.sem.chisq(n=seq(100, 1000, 50),
df=effect.res1$df, effect=effect.res1$delta, power=NULL)
```

```
pwr.curve2 <- wp.sem.chisq(n=seq(100, 1000, 50),
df=effect.res2$df, effect=effect.res2$delta, power=NULL)
```

```
pwr.curve3 <- wp.sem.chisq(n=seq(100, 1000, 50),
df=effect.res3$df, effect=effect.res3$delta, power=NULL)
```

```
pwr.curve4 <- wp.sem.chisq(n=seq(100, 1000, 50),
df=effect.res4$df, effect=effect.res4$delta, power=NULL)
```

plot() method is used to plot the power curves, e.g., *plot(pwr.curve1)* to plot power curve for the first hypothesis.

Produced with *plot()* method power curves are demonstrated in Fig. 3.

Power curve shows relationship between sample size and power. Power value shows how reliable is p-value. Say, power 0.9 safeguards 90% reliability, so that p-val-

ue is not incidental, that is only 10% to the chance for p-value to exceed the given. Power curves are built for p-value 0.05 as regular. We follow the suit. Usually sample size is telling enough given power higher 0.8. Power curves are ascending with gradual leveling off at larger sample sizes.

From the power curves of the case we judge the sample size to support error types 1 and 2 at arbitrary accepted levels 0.05 and 0.2 is 400 at least to test the influence of equilibrium price (p^*) on equilibrium quality (q^*). 600 sample size is needed to check for the influence of equilibrium price (p^*) on equilibrium comfort (c^*). Sample size of 600 is required to test hypothesis on informational noise about quality influences equilibrium value of quality. The most required is sample size to test fourth hypothesis on informational noise about comfort influences equilibrium value of comfort, reaching 1000.

DISCUSSION

The complete absence of power analyses for SEM of equilibrium is explained in part by the culprits of transition from theoretical balance models to SEM that can be processed with common statistical tools. SEM is prerogative for such transition as demonstrated in the paper. Dynamic equilibrium can be presumably rendered by state models, changing point models, antedependence models, etc. Whatever base formulation is considered next step is necessity to wrap it into SEM because as often as not equilibrium implies contemporaneous or lagged congruous move of many variables, each move described by partial derivate. So instead of single we have set of structurally related theoretical equations to pass on to SEM. We demonstrated how we can manage transition with example. Without doubt each case is different but some common tricks are there to use. Theoretical notions like patients' preferences, propensities, idiosyncrasy in response to treatment can be rendered with latent factors which are manageable within SEM. Other constituents of theoretical equation system describing equilibrium can be settled with additional beacons that greatly influence given constituents, like factor 1 in example unfortunately of latent nature but in other cases observable. This is our first try in the matter so experience is lacking, but hopefully the growing number of researches that can be enforced by theory with equilibrium support will yield further advancements, may be quite different from suggested in the paper. As for now, some recommendation to power analysis for SEM of equilibrium can be tentatively suggested as discussion points.

First of all, ad-hoc power analysis confined to particular structure (Fig.1) with no lee-way. Information on

parameters fed from other studies or expert opinions. The actual study data may not comply with ad-hoc values compromising derived sample size. Is it advisable to reassess sample size in the process of data collection? At least it is a possibility. Such power analysis is well known as ad-hoc. The new flexible approach is suggested by Ocheredko O. (2019) [5] that can be used to refine sample size estimates. It is implemented in R package *ltable* for categorical complex data and supports power analysis for simultaneous and consequential hypotheses testing.

Second, all four hypotheses should be tested jointly as ingrained in the same structure. The discussion point is what software is better for a purpose. Besides *WebPower*, other R packages can be used with even greater flexibility for ad-hoc power analysis for related tests, *lavaan* and *nimble* are paragon.

Third, given practicalities of data collection we may be unable to collect so many as power analysis suggests. It is usual situation that prompts to combine data with other sources or future data augmentation. Both options bring deduction closer to final. The point is that post-hoc power size calculus usually requires larger sample size. Therefore, ex post power evaluations of p-values are less optimistic, what was demonstrated in [5] and in manual to R package *ltable* with examples. It undermines deductions and findings of the accomplished study.

We also experience some uncertainty with Satorra & Sarris method. Caution is advisable for it compares two covariance matrices given main and zero hypotheses but it doesn't discriminate SEM structures. Several zero hypotheses with different SEM structures may result in very similar resultant matrices (discriminants/traces).

CONCLUSIONS

Power analysis for research in every field is of paramount importance. Without it we can't put credence to p-values of findings. The power analysis is of two branches, ad-hoc and post-hoc with different techniques applied. Yet it is far from perfection and there are constant debates about inconsistencies. The main is whether to get along with ad-hoc or rather with post-hoc. With ad-hoc analysis it's hard to process complex data, like that feeding to SEM. It's new ground that we are tentatively exploring in paper concerning SEM of equilibrium. The main challenge as we see it is the transition from theoretical balance models to SEM that can be processed with common statistical tools. SEM is prerogative for such transition as demonstrated in the paper.

Having hypotheses framed in solid theory gives hand to model elaboration and identification. SEM fits the purpose to a tea for its ability to incorporate structural dependencies and covariates along with directly unobservable factors. Power analysis is of particular reliability given complexity of construct that influence also possibility to test several hypotheses at once. Further post-hoc power analysis is needed to refine sample size for ad-hoc analysis can't comprise all specifications and possible measurement biases.

ABBREVIATIONS

MCMC – Monte Carlo Markov Chain
 OR\RR – odds ratio \ relative risk
 SEM – structural equations modeling
 r – correlation coefficient
 β – regression coefficient

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ORIGINAL ARTICLE

STUDY OF THE STRESS-STRAIN STATE OF THE MAXILLA DURING ORTHODONTIC TREATMENT OF DENTOGNATHIC DEFORMATIONS IN CHILDREN WITH CONGENITAL CLEFT LIP AND PALATE

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ABSTRACT

The aim: To create a three-dimensional simulation mechanical-mathematical model of the biomechanical system "Orthodontic appliance-maxilla", to study peculiarities of the stress-strained state of the maxilla.

Materials and methods: A simulation model of the biomechanical system "Orthodontic appliance-maxilla" was created using computed tomography (CBCT) data. Mathematical modeling was used to determine the stress-strain state of the simulation model.

Results: The patterns of changes in the stress state were determined and the values of deformation displacements in the structural elements of the biomechanical system "Orthodontic appliance-maxilla" were determined under a force stress of the orthodontic device with an amplitude of 50 N.

Conclusions: Simulation computer modeling of the stress-strain state of the "Orthodontic appliance-maxilla" system showed that activation of the kinematic mechanism of the appliance with a force of 50 N causes the emergence of a complex stress-strain state of bones. When the orthodontic appliance is activated, there is an asymmetry in the distribution of stresses by Mises between the right and left sides both for the appliance itself and for the maxillary bone tissue.

KEY WORDS: system "Orthodontic appliance-maxilla", distribution of stresses

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INTRODUCTION

The development of a pathological occlusion is facilitated by numerous etiological factors, which affect the child's craniofacial development [1, 2]. Defects in jaw development, in particular, largely determine the occlusion and the condition of the dentition. According to statistical data, 400-500 children in Ukraine are born each year with various types of congenital CLP [3-5], among which almost 94% of cases have dentognathic deformations [3, 5-9]. The variety of clinical manifestations of malocclusion in children with congenital CLP contributes to the development and implementation of new methods of their diagnostics and treatment [9, 10].

Orthodontics is a branch of dentistry, which is deeply connected with engineering and biomechanics [11]. In recent decades, scientists thoroughly studied the peculiarities of the mechanical interaction of orthodontic devices and biological tissues using the methods of mechanical and mathematical modeling. For example, they investigated the issues of the shape of dental arches during orthognathic occlusion [12], mechanical

and mathematical modeling of the process of orthodontic treatment of dentognathic deformations using pre-orthodontic trainers [13], the process of treatment of dental arch defects [14], tooth rotations [15], open occlusion [16], etc. Other studies include investigations of orthodontic forces in devices in the treatment of mesial occlusion [17] and sagittal anomalies of occlusion [18]. The effect of the forces applied to the tooth during orthodontic treatment after 3D scanning of the oral cavity was evaluated on geometric models [19].

The works of scientists outline a number of problems of computer modeling of biomechanical systems based on CBCT data [20, 21], analysis of a simulated three-dimensional model of the system "Bone tissue-dental implant-suprastructure" [22], biomechanical interaction in fixed partial prostheses [23], simulated computer modeling in maxillofacial surgery [24, 25], a study was conducted on the development of three-dimensional (3D) modeling of finite-elements for predicting tooth movement in the treatment of occlusal deformities [26] and diastema [27], three-dimensional monitoring

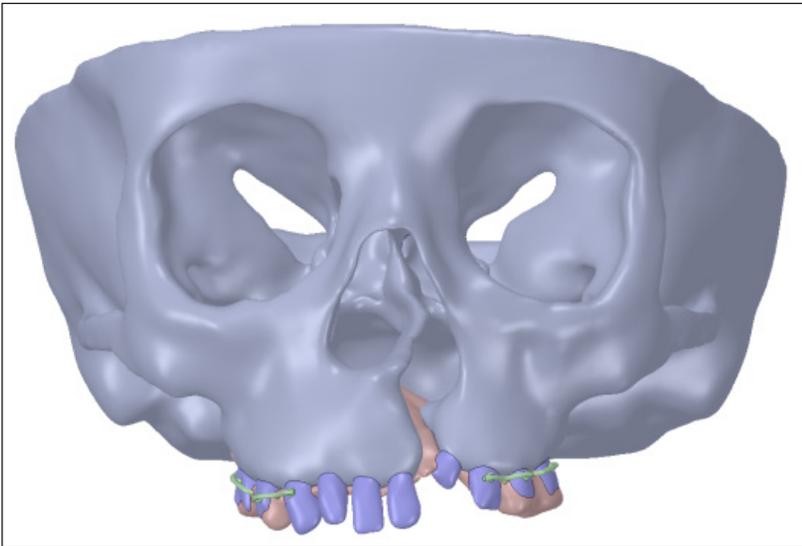


Fig. 1. Imitation model of the maxilla in a child with congenital unilateral CLP



Fig. 2. A sample of a removable orthodontic appliance used in the orthodontic treatment of a patient with a mixed dentition period with congenital unilateral CLP

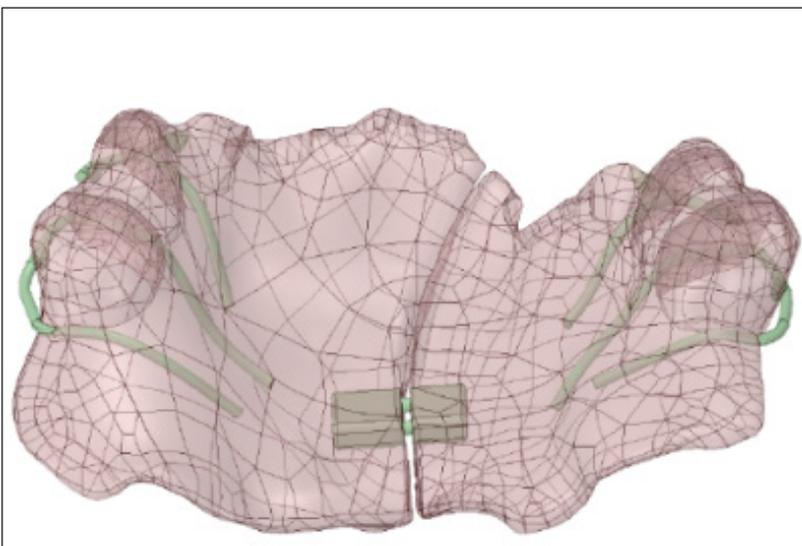


Fig. 3. 3D simulation model of the orthodontic appliance

of root movement during orthodontic treatment was described [28]. A mathematical model was developed for assessing the intensity of the force on elements of the bracket system [10]. Despite such a large number

of studies on biomechanical systems in orthodontics, there are no studies of the stress-strain state of the maxilla in children with congenital CLP during orthodontic treatment.

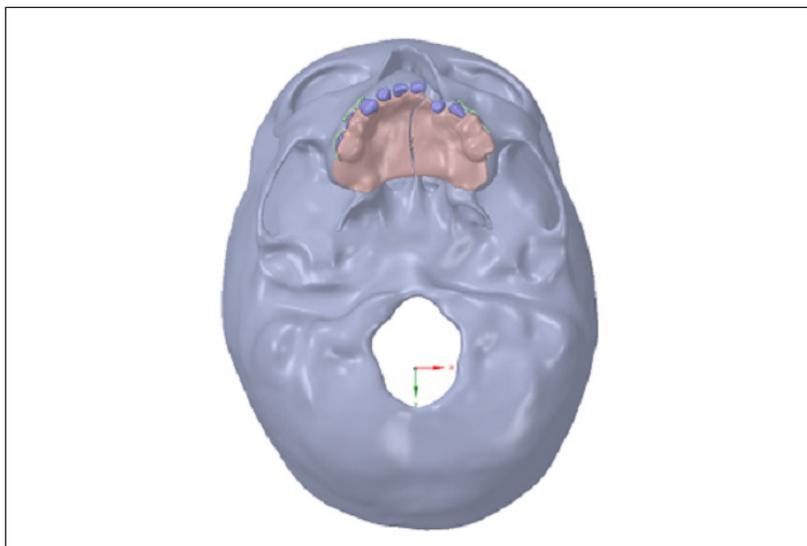


Fig. 4. Imitation model of the maxilla in a child with congenital unilateral CLP with an attached orthodontic device

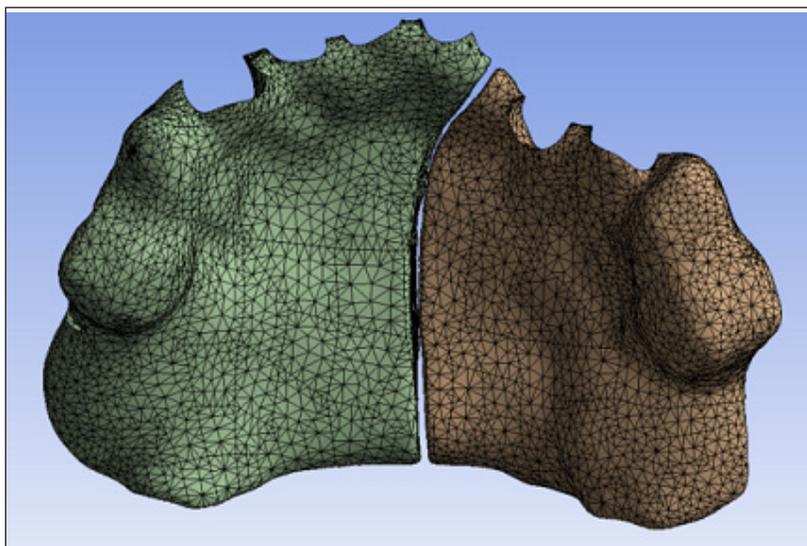


Fig. 5. Discrete model of an orthodontic appliance



Fig. 6. Discrete model of the maxilla of a child with congenital unilateral CLP with an attached orthodontic device

THE AIM

To create a three-dimensional simulation mechanical-mathematical model of the biomechanical system "Orthodontic appliance-maxilla" in the course of orthodontic treatment

of a patient in the mixed dentition period with congenital unilateral CLP, to study peculiarities of the stress-strained state of the maxilla under the action of static loads caused by the activation of the orthodontic appliance.

Table I. Models of materials of the biomechanical system

Structural elements	Yung's module, E, MPa	Poisson's ratio, ν	Strength limit
Cortical layer of maxillary bones	12650	0,25	120
Cancellous layer of maxillary bones	470	0,27	8
Metal retainers (clasps)	200000	0,3	640
Base of the orthodontic appliance	2500	0,3	80

Table II. Maximum equivalent by Mises stresses in the elements of the system "Orthodontic appliance-maxilla", permissible stresses and safety factors for the case of a quasi-static load

Structural elements of the biomechanical system	$\sigma_{\text{экв}}$ equivalent stresses, MPa	σ_b , strength limit, MPa	$[\sigma]$, permissible stresses, MPa	η , safety factor
Maxillary bones	10.3	120	80.0	7.8
Orthodontic appliance	5.8	80	53.3	9.2
Metal retainers (clasps)	1.3	640	426.7	252.8

MATERIALS AND METHODS

The biomechanical behavior of the maxilla of a patient with unilateral CLP during orthodontic treatment was studied in a model experiment using the finite-element method. During the experiment, a simulation computer model of the stress-strain state of the "Orthodontic appliance-maxilla" system was created under static load conditions.

A three-dimensional virtual model of midface bones was built in the Mimics Medical 25.0 (Materialise, Belgium) based on CBCT data of the skull of patient P., 6.5 years old (mixed dentition period) with congenital unilateral through CLP at the stage before eliminating the residual defect of the hard palate.

CBCT data presented as a series of DICOM files were imported into Mimics software for further segmentation, during which cortical and cancellous layers of the maxilla were identified [24]. Separation of these biomechanically heterogeneous volumes of the maxilla was performed according to the radiological density of the corresponding biological structures (Fig. 1).

Digital data for the spatial geometry of a typical medical device consisting of a base, a kinematic mechanism (orthodontic screw) and metal retainers (clasps) presented in STL format were used as a model of the appliance (Fig. 2). At the same time, the simulation model was represented by two fragments of the base of the appliance connected in the area of the screw and supporting external retainers of different spatial configuration (Fig. 3).

The surface models created using Boolean operations were combined into multi-component assemblies and imported into Ansys 12.1 software (Fig. 4).

For each element of the model, volumes and a finite-element mesh were created followed by the assignment of the corresponding material properties. The discretization of all structural elements of the simulation model of the appliance was carried out in

the semi-automatic mode of ANSYS 12.1 [30] using 10-nodal pyramidal finite-elements (FE) SOLID187 with 731865 nodes (Fig. 5).

A discrete model of the maxilla of a child with congenital unilateral CLP with an attached orthodontic device was built with the maximum size of finite-elements (FE) no more than 1.5 mm, which had 1 281 160 CE Solid187 with 731 865 nodes (Fig. 6, 7).

The basic mechanical properties of the bone, plastic and steel elements of the appliance were set according to results of experimental studies and available technical standards [24]. The base of the orthodontic appliance was assigned with properties corresponding to the material – plastic for the prosthesis bases. To simplify calculations, all materials were considered homogeneous, linearly elastic and isotropic.

The mechanical properties of structural elements of the simulation model are given in Table I [29].

To determine the kinematic boundary conditions, the model of midface bones was rigidly fixed blocking all types of linear and angular movements. Clasps of the orthodontic device were attached to the corresponding teeth of the maxilla using a rigid type of contact connection.

Calculated force vectors with an amplitude of 50 N were used to model the axial movements of parts of the appliance from 360° angular movements, which corresponded to one rotation of the screw. They served as initial values for determining the force loads of the corresponding surfaces in the developed calculation scheme for contacting deformable bodies of the maxilla and clasps of the orthodontic device (Fig. 8).

The total strain of the system was determined (the maximum movement of the nodes during deformation), the maximum values of the main and equivalent stresses, as well as the patterns of the distribution of stresses and strains (demonstrated in the form of color

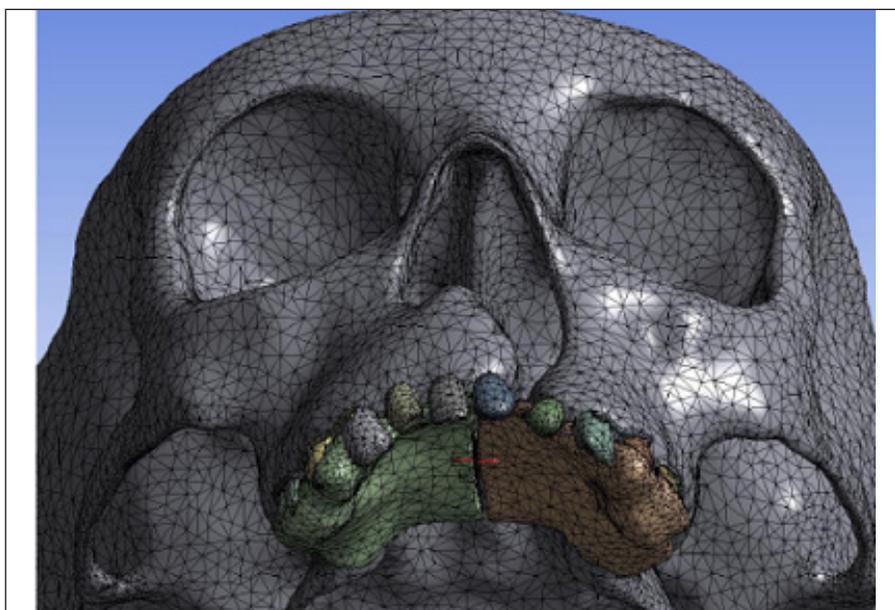


Fig. 7. Visualization of a discrete model of the maxilla of a child with congenital unilateral CLP with an attached orthodontic device

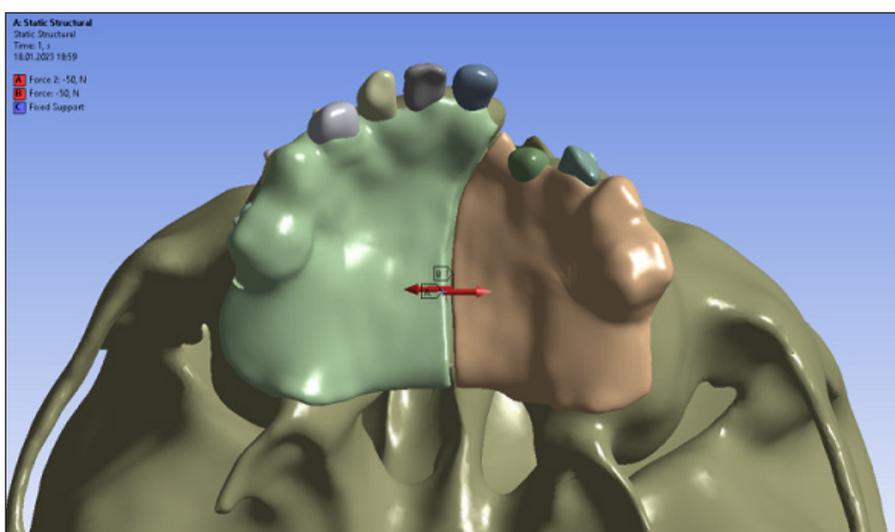


Fig. 8. Calculation scheme of the maxilla with an attached orthodontic device

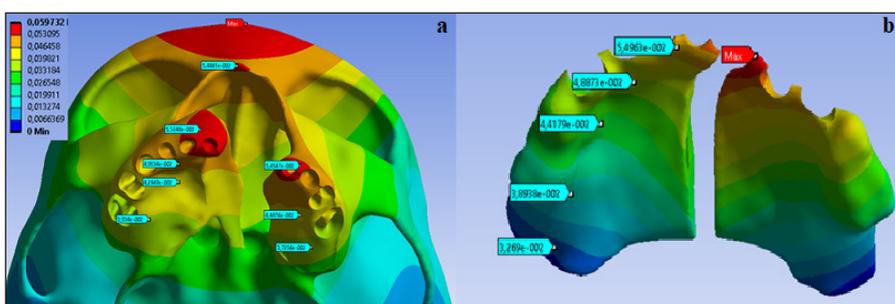


Fig. 9. Fields of displacement in mm of the maxilla (a) and the orthodontic device (b) under a force load, $P=50\text{ N}$

gradients or vector graphics for the cortical, cancellous layer of the bone and appliance). The strength and reliability of the “Appliance-bone” system was evaluated by the maximum value of the equivalent stresses in the structural elements and bone tissue of the maxilla by comparing them with the maximum permissible values for different types of material known from the literature.

RESULTS

The results of the numerical experiment are represented by fields of displacement (in mm) of the maxilla (Fig. 9 a) and the orthodontic device (Fig. 9 b) under a force load, $P=50\text{ N}$, by the fields of equivalent by Mises stresses of the maxilla (Fig. 10 a - c) and the orthodontic device (Fig. 10 d) at the specified level of force loads and the direction of movement of the

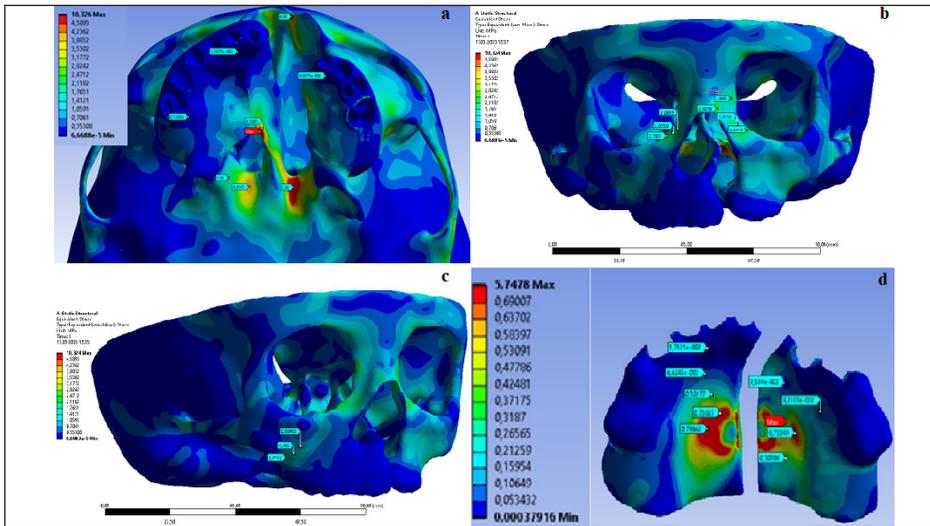


Fig. 10. Fields of equivalent by Mises stresses of the maxilla (a - c) and the orthodontic device (d) under a force load, $P=50$ N

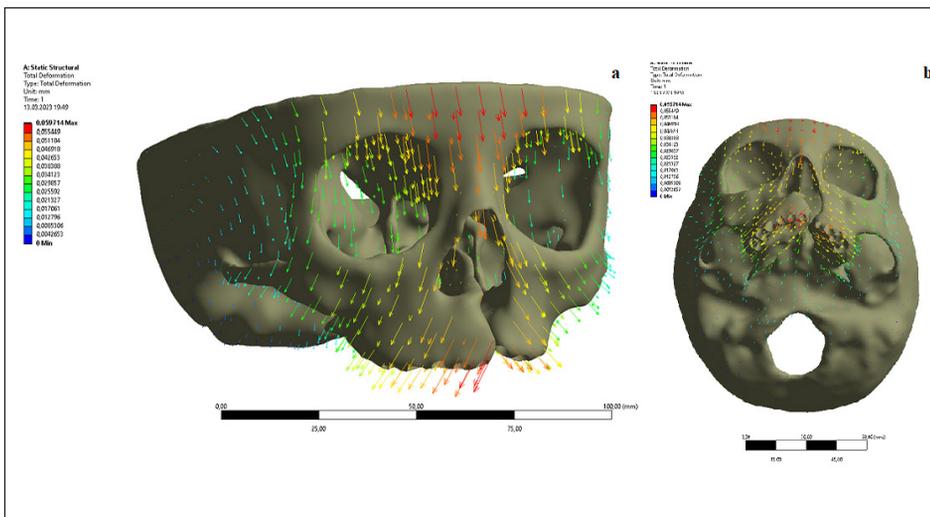


Fig. 11. The direction of displacement of the model nodes under the given conditions of the force load (a, b)

nodes of the model under the specified conditions of the force load (Fig. 11 a, b).

Analysis of the obtained data determined the magnitudes of the deformation displacements of structural elements of the biomechanical system for the given amplitude of the force load of the orthodontic appliance, which have the greatest clinical importance.

The "Orthodontic appliance-maxilla" system was in a complex stress-strained state, which implied the presence of stretching, compression, bending and torsional deformations. The maxilla underwent expansion, most pronounced in the area of the alveolar process in the cleft zone. For example, along the anterior edge of the cleft, the maximum displacements q_{\max} were 0.053 mm. On the other hand, in the posterior part of the cleft, they did not exceed 0.037 mm. On the edges of the surface of parts of the appliance with a screw, q_{\max} was in the range from 0.039 mm to 0.059 mm. The non-homogeneity of the deformations of the orthodontic apparatus itself was caused by the constructive location of the screw relative to the base of the device. In addition, the degree of deformation directly depended on the spatial location of the device on the maxilla.

Force loads were transmitted from the orthodontic appliance through the teeth to which it was attached on the maxilla tissue, while the extreme values of equivalent by Mises stresses in its cortical layer reached $S_{\text{âââ}} = 10.3$ MPa. The equivalent by Mises stresses in the orthodontic wire turned out to be quite small; their values were in the range from 0.28 MPa to 1.3 MPa.

At the same time, stress distribution in the bone tissue was not uniform: the maximum gradients of equivalent stresses were observed in the area of the osseous nasal septum, horizontal plates of the palatine bone, in the vicinity of the pterygoid-maxillary junction, in the area of the zygomatic-alveolar process and the frontal process of maxilla. Through the system of vertical and horizontal counterforces of the midface, stresses from the orthodontic appliance were redistributed and transferred to the structures of the cranial base and vault.

The greatest stresses (from 5 to 10.3 MPa) were observed in the area of the osseous nasal septum and the posterior third of the hard palate. Tensile forces capable of stimulating appositional bone growth prevailed in these zones. On the other hand, the naso-frontal and

zygomatic-alveolar counterforces underwent predominant bending and displacement, and the value of equivalent by Mises stresses in the zones of their maximum concentration ranged from 1.5 to 3.5 MPa. At the same time, asymmetry in the distribution of stresses, which were 18-24% higher around the small fragment of the maxilla, was observed.

The stress distribution in the base of the orthodontic appliance was uneven; the areas around the screw took the greatest loads. The maximum gradients of equivalent by Mises stresses were observed at a distance of one third of the width of the appliance, where S_{eq} values in these zones ranged from 0.73 MPa to 0.79 MPa, while their maximum values reached 5.8 MPa.

An approximate estimate of the strength and permissible values for this type of force loads in the elements of the biomechanical system "Orthodontic appliance-maxilla" was calculated (Table II). Permissible stresses for materials are given with a safety factor of 1.5.

The analysis showed that the safety factor $\eta > 1$ was established for all structural elements of the studied biomechanical system. The bearing capacity of this biomechanical system under a force load with an amplitude of 50 N is determined by the strength of maxillary bones with a safety factor of $\eta = 7.8$, which corresponds to a 360° rotation of the kinematic mechanism rod and an axial movement of 1 mm.

DISCUSSION

In view of account the data on the prevalence of congenital CLP [3-5] and the percentage of dentognathic deformations with a variety of clinical manifestations [3, 5-9], it is important to develop and implement new methods of their diagnosis and treatment. The study of the features of the biomechanical behavior of systems that include the orthodontic structure and the maxillofacial apparatus in experimental and theoretical studies to determine the rigidity of orthodontic devices is the basis of high-quality complex rehabilitation of patients with congenital defects.

Experimental and theoretical studies are based on mechanical and mathematical modeling of the stages of orthodontic treatment of dentognathic deformations [9, 10, 12-19]. The analysis of the interaction of parts of biomechanical systems, the study of the stress-strain state of the dentognathic apparatus, the study of points of resistance during orthodontic treatment, the issue of the process of treating defects of the dentition in children, tooth rotations, open bite are fully covered in modern scientific literature [10, 20-28]. Ukrainian and foreign scientists have covered the issue of the expansion of the upper jaw [13-18, 31]. However, the

mentioned studies covered the spectrum of the stress-strain state of the dentognathic apparatus without congenital malformations. Taking into account the anatomic-topographical and functional features of the unfused upper jaw [3, 5, 8], the data can't be fully interpreted in children with congenital CLP.

Taking into account the specifics of dentognathic deformations in this category of patients, the question of the regularity of changes in the stress-strain state and the determination of the magnitude of deformation displacements in the structural elements of the biomechanical system "Orthodontic appliance-maxilla" under the force stress of the orthodontic device is modern and relevant.

Since scientific sources do not pay attention to this issue, the results of numerical experiments conducted for the first time in children with congenital defects of the maxillofacial area can be used to prognostication the treatment strategy using an orthodontic device of a certain configuration. Verification of the distribution of force response values of orthodontic devices in the process of their deformation allows obtaining new data on the magnitude of external loads on the jawbones.

Prospects for further research should be aimed at determining the radiological density of bone tissues of the maxillofacial area in children with congenital CLP in defined resistance zones, where the stress distribution is not uniform.

CONCLUSIONS

According to results of simulation computer modeling of the stress-strain state of the "Orthodontic appliance-maxilla" system, the activation of the kinematic mechanism of the device with a force of 50 N causes the emergence of a complex stress-strain state of the midface bones, which include stretching, compression, bending and torsional deformations. The largest displacements of the model nodes are observed on the alveolar process of the maxilla in the anterior part of the cleft, which are 30% larger than in the posterior part.

Equivalent by Mises stresses in the maxillary bone tissue are unevenly distributed. Their maximum gradients were observed in the area of the osseous nasal septum and the posterior third of the hard palate (5-10.3 MPa), the frontal process of maxilla, the zygomatic-alveolar crest, and the pterygoid-maxillary junction (1.5 to 3.5 MPa).

When the orthodontic appliance is activated, there is an asymmetry in the distribution of stresses between the right and left sides both for the appliance itself and for the maxillary bone tissue (maximum stresses were 18-24% higher on the small fragment than on the large one), which was due to the structural arrangement of the kinematic mechanism

relative to the base of the device and the peculiarities of the spatial location of the device on the maxilla.

When the appliance is activated with a force of 50 N, which corresponds to a 360° rotation of the kine-

matic mechanism rod, the stresses in the base of the device and bone tissue do not exceed the maximum permissible values. The safety factor η is 9.2 for the device and 7.8 for the bone tissue.

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ORIGINAL ARTICLE

THE POTENTIAL RENOPROTECTIVE EFFECT OF TILIANIN IN RENAL ISCHEMIA REPERFUSION INJURY IN MALE RAT MODEL

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ABSTRACT

The aim: To determine whether Tilianin (TIL) may have Nephroprotective effects on bilateral renal IRI in rats by analyzing kidney function biomarkers U and Cr, inflammatory cytokines like TNF α and IL-1 β , antioxidant marker total anti-oxidant Capacity (TAC), anti-apoptotic markers caspase-3, and histopathological scores.

Materials and methods: 20 rats divided into even 4 groups as: Sham group: Rats underwent median laparotomies without having their ischemia induced. Control group: Rats had bilateral renal ischemia for 30 minutes, followed by 2 hours of reperfusion. Vehicle group: 30 minutes prior to the onset of ischemia, rats were given a pretreatment of corn oil and DMSO. Tilianin treated group: Rats administered Tilianin 5 mg/kg for 30 min prior to ischemia induction, then IRI.

Results: The study found that the serum levels of TNF, IL-1, caspase-3, urea and creatinine, as well as TNF and creatinine in the Tilianin group were significantly lower than those of the control and vehicle groups. On the other hand, it revealed that TAC levels are remarkably higher in the Tilianin group than they are in the control and vehicle groups.

Conclusions: This study concluded that Tilianin have a Nephroprotective effect via multiple impacts as anti-inflammatory, anti-apoptotic, and anti-oxidant agents.

KEY WORDS: Tilianin, renal ischemia reperfusion injury, male rat model

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INTRODUCTION

A temporary decrease in blood flow that carrying oxygen to the renal then followed by reperfusion, referred to a renal ischemia reperfusion injury (IRI). During IRI, kidney tissue injury promotes excess production of reactive oxygen species (ROS), which induces oxidative stress. During reperfusion time, restoration of blood flow generates even greater generation of ROS that ends up with apoptosis and cell death [1]. A clinical state known as acute kidney injury (AKI), which is marked by fast renal failure and high mortality rates, is developed as a result of IRI involving renal tissues. Renal IRI pathology involves a number of pathogenic processes, including the generation of reactive oxygen species and the activation of various inflammatory mediators such as adhesion molecules and a number of cytokines [2]. When compared to other organs, there are several notable differences in the hemodynamics and oxygenation of the kidneys. 20 percent of the cardiac output is delivered to the kidneys, but while 100 percent of this reaches the cortex, only 15 percent reaches the medulla [3]. Unquestionably, the inflammatory response plays a significant role in the etiology of IRI. Numerous experimental studies have

demonstrated that inhibiting inflammatory responses lowers renal IRI while maintaining renal function [4]. It has been demonstrated in numerous organs that the microvascular and parenchymal tissue damage that results from ischemia and reperfusion is predominantly caused by reactive oxygen-free radicals. As a result of a reduction in the synthesis of antioxidant enzymes, post-ischemic reperfused tissue has more damage caused by free radicals [5]. A cytokine called tumor necrosis factor alpha (TNF α) has pleiotropic effects on several cell types. TNF- α has been identified as a critical regulator of inflammatory reactions and is known to have a part in the genesis of a number of inflammatory and autoimmune diseases [6]. Additionally, it induces the production of additional cytokines since IL-1 β , a key proinflammatory cytokine, has a variety of purposes in various organs and diseases [7]. Despite being multifaceted, IRI pathophysiology involves inflammation and oxidative stress. During the ischemia phase, a lack of oxygen and nutrients results in the accumulation of hypoxanthine, and induction of pro-inflammatory cytokines. The reperfusion phase causes a rise in iNOS and NOXs in endothelial cells, and the influx of neutrophils [8].

One of the primary signs of oxidative stress is malondialdehyde (MDA), which is formed upon breaking down of lipid peroxyl radicals. The only reliable method for determining an organism's antioxidant capability is through TAC measurement [9]. A decrease in kidney tissue TAC levels was noted during renal IRI [10]. Apoptosis is the process through which a cell ceases to divide and develop in favor of an action that finally results in the cell's controlled death without discharging its contents into the immediate environment [11]. Apoptosis occurs as a result of excessive reactive oxygen species (ROS) generation that causes oxidative stress, which in turn triggers lipid peroxidation and ultimately results in cell death during the process of renal ischemia reperfusion damage [12]. When a cell undergoes apoptosis, caspase-3, the final caspases in the cascade, is activated by both internal and extrinsic mechanisms [13]. Caspase-3 has been extensively examined among the several caspases proteins, and it has been suggested that it plays a major factor in renal dysfunction [14]. A protein kinase signalling molecule called extracellular signal-regulated kinase (ERK) belongs to the MAPK family along with p38 MAPKs, extracellular signal-regulated kinase 5 (ERK5), and c-Jun N-terminal kinases (JNKs). The phosphorylation processes that trigger these enzymes, which are a component of the mitochondrial route for apoptosis, amplify and send signals from the cell membrane to the nucleus. Growing data suggests that the MAPK protein family is essential for ROS-mediated apoptosis [15]. Among the three recognized MAPK signalling pathways, ERK-associated intracellular signal transduction pathways are considered as traditional MAPK pathways. Activation of the P38MAPK and ERK1/2 signal transduction pathways is known to regulate cell growth and differentiation, but accumulating evidence suggests that these pathways also play a role in cell death [16]. An immediate early gene called transcription factor-early growth response-1 (EGR1) is involved in the processes of growth, differentiation, apoptosis, and wound healing [17]. EGR1 was found in the proximal tubule of kidney disease patients and was activated by hypoxic stimuli. EGR1 silencing could improve diabetic kidney disease and prevent renal fibrosis and inflammation [18].

Tilianin, also known as acacetin-7-glucoside, is an active flavonoid glycoside that is obtained from several medicinal plants, most notably *Dracocephalum moldavica*. It was highlighted for a wide range of biological activities, including anti-diabetic characteristics [19], anti-inflammatory, antioxidant, and anti-depressant effects, Cardioprotection [20, 21], and neuroprotection. Tilianin suppresses cell

death through the mitochondrial route, making it a potential therapeutic treatment for ischemia reperfusion-induced AKI. This impact is achieved by reducing ERK pathway activation and downregulating EGR1 expression [18].

THE AIM

The aim of this research is to determine whether Tilianin (TIL) may have nephroprotective effects on bilateral renal IRI in rats by analyzing kidney function biomarkers U and Cr, inflammatory cytokines like TNF α and IL-1 β , antioxidant marker total anti-oxidant capacity (TAC), anti-apoptotic markers caspase-3, and histopathological scores.

MATERIALS AND METHODS

ANIMAL PREPARATION, TREATMENT, AND SACRIFICE

Adult Sprague-Dawley rats were used in this study weighing between 200 and 350 g at ages 20 to 24 weeks. They were acquired from the Ministry of Health/Center of Control and Pharmaceutical Research/Baghdad and housed in the animal home at the Animal Resources Center/College of Sciences/University of Kufa. The rats were fed a normal diet made up of food and tap water. All rats were included in the study once the Institutional Animal Care and Use Committee (IACUC) at the University of Kufa gave its consent.

STUDY DESIGN

The rats were randomly separated into an even 4 groups (each group had five rats) for the case control study's case study design:

- Sham group: Rats experienced median laparotomy for around 2 hours and 30 minutes, but with no ischemia induction;
- Control group: Rats experienced bilateral renal ischemia for 30 min, then initiate reperfusion through restoring renal blood flow for 2 hours [22];
- Vehicle group: Rats administered intraperitoneal injection of mixture of corn oil and DMSO (Medchemexpress/USA) 30 min before ischemia induction. Then rats experienced 30 min bilateral renal ischemia and 2 hours' reperfusion [22];
- Tilianin treated group: Total of five rats administered intraperitoneal injection of Tilianin 5 mg/kg [23] 30 min [24] before ischemia induction. Then after receiving anesthesia, the rats experienced 30 min bilateral renal ischemia and 2 hours' reperfusion [22].

EXPERIMENTAL STUDY MODEL

Before the experiment, rats must be weighed, for which 100 mg/kg ketamine and 10 mg/kg xylazine are injected intraperitoneally to provide anesthesia. After the rats were fixed with stickers, their chest and abdominal hair were shaved, and the full sedation were ensured. Through a midline laparotomy incision, the intestine was withdrawn, exposing the abdomen and both renal pedicles. The renal pedicles were isolated for the bilateral ischemia model by securing non-trauma micro vascular clamps around the renal arteries and veins [25]. After a few minutes, the color of the kidneys changes from red to dark purple, and light stains start to develop on the surface, indicating that blood flow is being blocked. The pedicles were released from the clamps after 30 minutes, allowing renal blood flow to be restored, and the dark purple color of the kidneys changed to pale red [31], signaling the start of the two-hour reperfusion phase [25]. The kidney was repositioned and the abdominal cavity incision was stitched with three interrupted sutures. Then euthanasia is performed by deep anesthesia. Finally, blood and tissue samples were gathered for the study. It has been demonstrated that ischemia lasting more than 20 minutes causes kidney injury [27].

PREPARATION OF TILIANIN

The pure powder of Tilianin was purchased from Medchemexpress, USA Company. Molecular Formula: $C_{22}H_{22}O_{10}$. Chemical name: acacetin-7-O- β -D-glucopyranoside. CAS Number: 4291-60-5. Purity: > 98% (HPLC). Physical description: solubility is more than 2.08 mg/mL (4.66 mM). Administer 2.403 ml/kg intraperitoneally, prepared by dissolving 2.08 mg of Tilianin in a mixture of 10% DMSO and 90% corn oil according to instructions leaflet of manufactured company and prepared immediately before use (Med Chem Express company instructions). The dose of drug that was used is 5mg/kg of rat weight intraperitoneally [23].

PREPARATION SAMPLES OF BLOOD FOR DETERMINING OF KIDNEY FUNCTION PARAMETERS

After two hours of reperfusion, the surgery was completed, and blood was immediately drawn from each rat's heart (3.5-5 ml). The blood sample was placed in a gel tube that had been pre-labeled and left untreated for 30 minutes at a temperature of 37°C. The serum required for determining urea and creatinine was then

produced by centrifuging each gel tube at 3000 rpm for 10 minutes [28].

PREPARATION OF TISSUE FOR MEASUREMENT OF TNF-A, IL1-B, AND CASPASES 3 BY ELISA, AND MEASUREMENT OF TOTAL ANTI-OXIDANT CAPACITY BY COLORIMETRIC METHOD

At the end of reperfusion period, the left kidney was removed and rinsed with ice-cold isotonic solution 0.9% to eliminate any blood clots before being divided into two parts. One part undergo homogenization with a high intensity ultrasonic liquid processor in a percent of 1:10 (w/v) phosphate buffered saline that contain 1% Triton X-100 and a protease inhibitor cocktail. Then the homogenate undergo centrifugation at 14000 rpm for 20 min at a temperature 4°C [29]. Thereafter, the supernatant is collected for determination of TNF- α , IL1- β , and Caspase3 by ELISA technique as well as TAC measurement through colorimetric method.

SAMPLING OF TISSUE FOR HISTOPATHOLOGY ANALYSIS AND GRADING THE SCORE DAMAGE

The remaining left renal tissue was fixed in 10% formalin, dehydrated in a series of alcohols, cleaned in xylene, and then embedded in paraffin to create the paraffin block. Once fixation was complete, a score evaluation utilizing light microscopy was done by a researcher who was unaware of the experimental treatment groups. Renal tubule injury is indicated by tubular epithelial swelling, brush boundary loss, vacuolar degeneration, and cast formation [30]. A magnification of X100 and X400 was used to determine renal damage. The percentage of renal tubular damage was calculated from the score of histological alterations in the kidney tissue segment:

Score 0, represents normal;

Score 1, represent < 25% of damage tubules;

Score 2, represent 26%-50% of damage tubules;

Score 3, represent 51% -75% of damage tubules;

Score 4, represent 76% -100% of damage tubules [31].

STATISTICAL ANALYSIS

According to GraphPad Prism version 7, the experimental results were statistically analyzed using Tukey multiple comparisons. A one-way analysis of variance (ANOVA) was used to determine the significance of group differences. P value \leq 0.01 was regarded as statistically significant. The values were given as mean \pm standard errors of the mean (SEM).

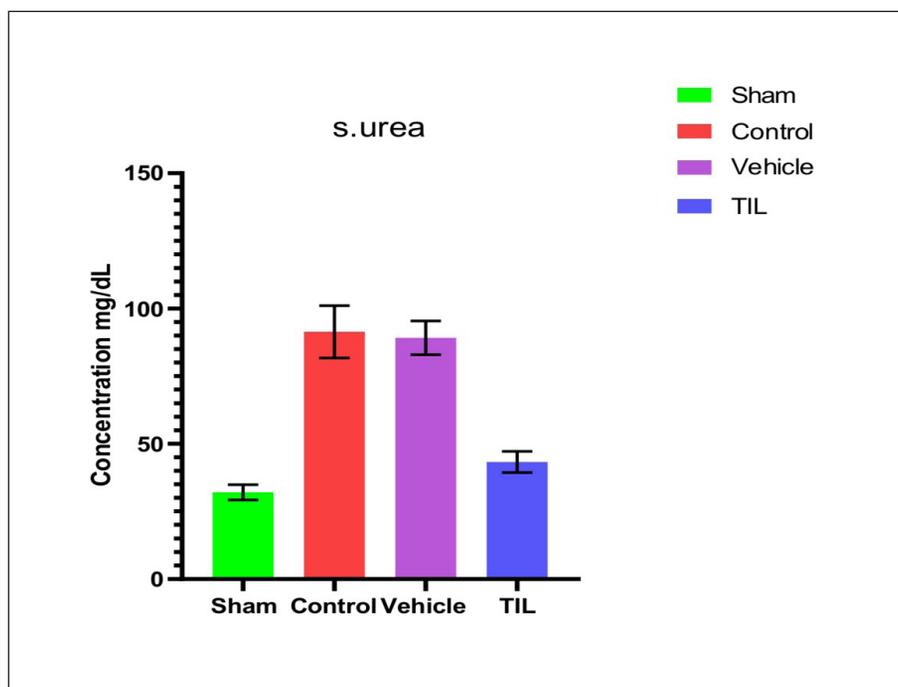


Fig. 1. Mean serum urea (mg/dl) level of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p -value ≤ 0.01 ; TIL group vs. Control & Vehicle groups: p -value ≤ 0.01 ; TIL: Tilianin.

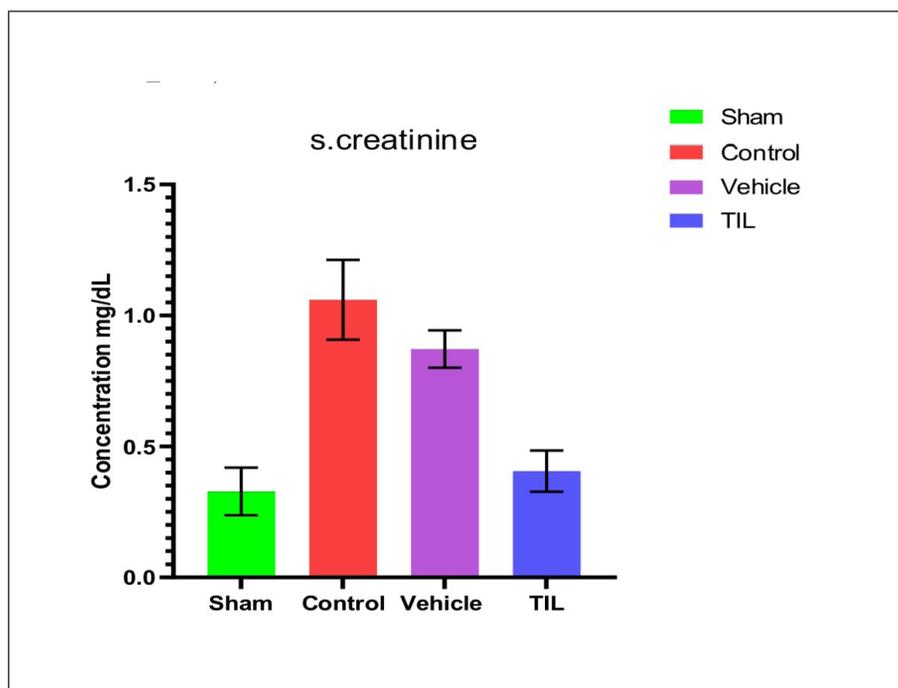


Fig. 2. Mean serum creatinine (mg/dl) level of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. P value ≤ 0.01 ; TIL group vs. Control & Vehicle groups: p -value ≤ 0.01 ; TIL: Tilianin.

RESULTS

Effects of IRI and Tilianin on kidney function parameters (blood urea nitrogen and serum creatinine)

The results of the current study showed that serum levels of urea (U) and creatinine (Cr) were significantly elevated ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in serum levels of urea and creatinine.

Mean serum U and Cr level as it shown in figure 1 and figure 2, respectively.

EFFECTS OF IRI AND TILIANIN ON INFLAMMATORY MARKERS TNF-A AND IL1-B

The results of the current study showed that renal tissue levels of Tumor Necrosis Factor (TNF- α) and Interleukine-1 β (IL1- β) were significantly elevated ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when

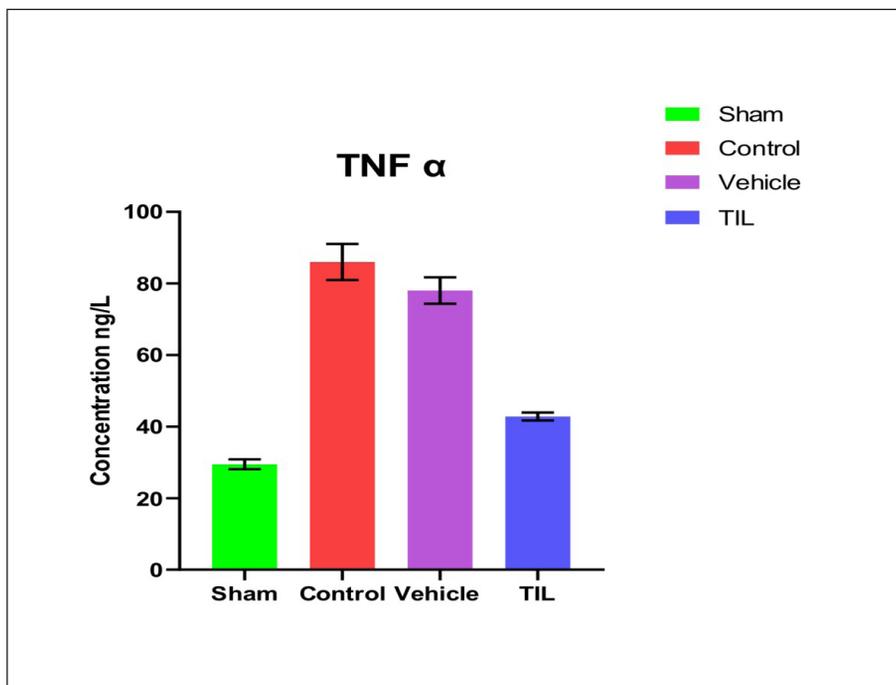


Fig. 3. Mean level of TNF α in renal tissue (ng/l) of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value \leq 0.01; TIL group vs. Control & Vehicle groups: p-value \leq 0.01; TIL: Tilianin.

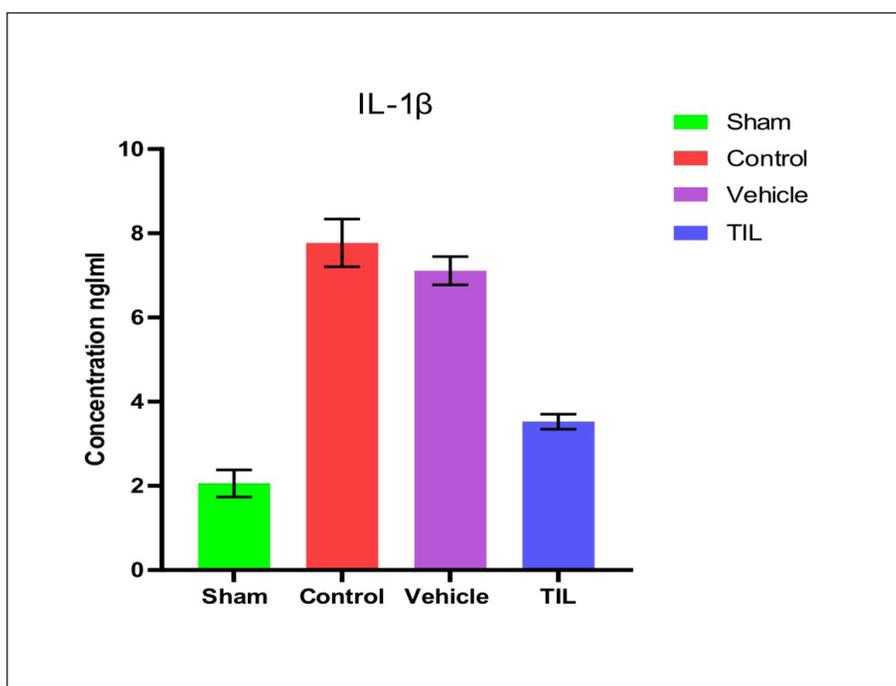


Fig. 4. Mean serum IL-1 β (ng/ml) level of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p-value \leq 0.01; TIL group vs. Control & Vehicle groups: p-value \leq 0.01; TIL: Tilianin.

compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in renal tissue levels of TNF- α , and IL1- β . Mean tissue TNF- α and IL1- β level are shown in figure 3 and figure 4 respectively.

control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) decrease in renal tissue levels of caspase-3. Mean tissue caspases 3 level (Fig. 5).

EFFECTS OF IRI AND TILIANIN ON APOPTOTIC MARKER (CASPASE-3)

The results of the current study showed that renal tissue level of caspase-3 were significantly elevated ($p \leq 0.01$) in the

EFFECTS OF IRI AND TILIANIN ON TAC

The results of the current study showed that renal tissue level of total anti-oxidant capacity (TAC) were

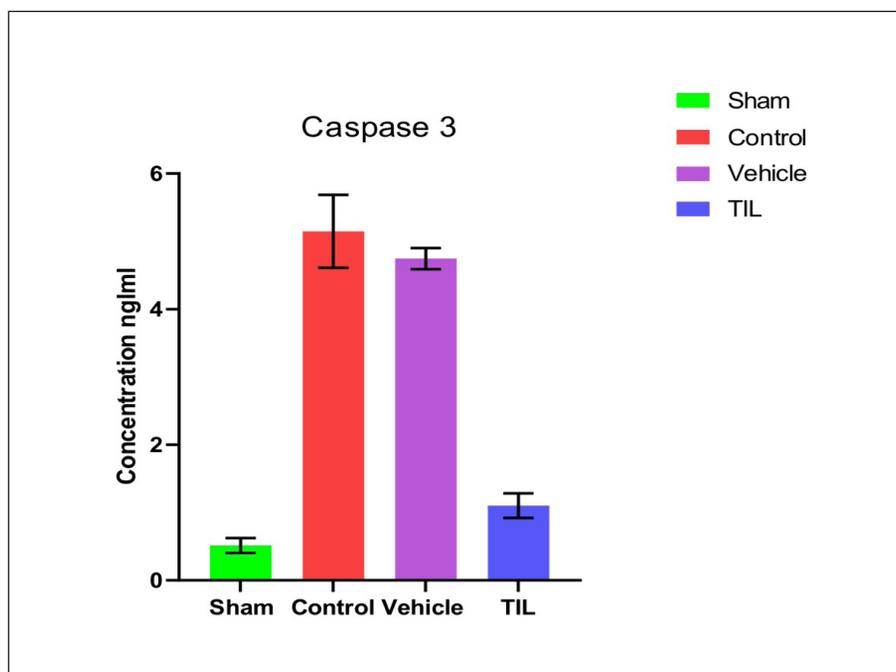


Fig. 5. Mean serum caspase-3 (ng/ml) level of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p -value ≤ 0.01 ; TIL group vs. Control & Vehicle groups: p -value ≤ 0.01 ; TIL: Tilianin.

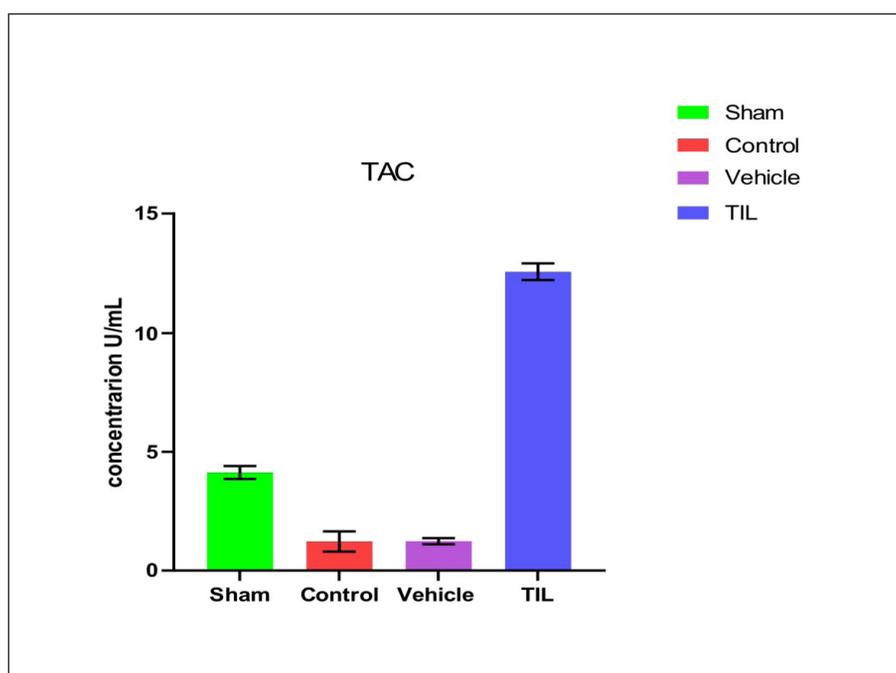


Fig. 6. Mean serum TAC (U/ml) level of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p -value ≤ 0.01 ; TIL group vs. Control & Vehicle groups: p -value ≤ 0.01 ; TIL: Tilianin.

significantly reduced ($p \leq 0.01$) in the control and vehicle groups compared to the sham group. However, there was no significant ($p \geq 0.01$) difference between the control and vehicle groups when compared. However, when compared to the levels in the control and vehicle groups, the Tilianin-treated group demonstrated a substantial ($p \leq 0.01$) increase in renal tissue levels of TAC. Mean tissue TAC level (Fig. 6).

HISTOPATHOLOGICAL EXAMINATION

The damage score and the histology results are displayed in figure 7 and figure 8 at the end of the study.

The kidney tissue cross section from the sham group displayed a normal renal structure, glomerulus, and kidney tubules (Fig. 8A). As it shown, the renal tissue cross section from the control group (Fig. 8B), on the other hand, showed aberrant renal structure and severe kidney damage, with the renal tubules being damaged to an extent of 80% and showing enhanced eosinophilia, vacuolated epithelium, and Eosinophilic cast; interpreted in high severity score (severity score mean=4). The kidney tissue of the vehicle group was cross-sectioned, revealing injured kidney structure and severe renal injury (damage of about 80%), including renal tubules dilatation, loss of the brush border, in-

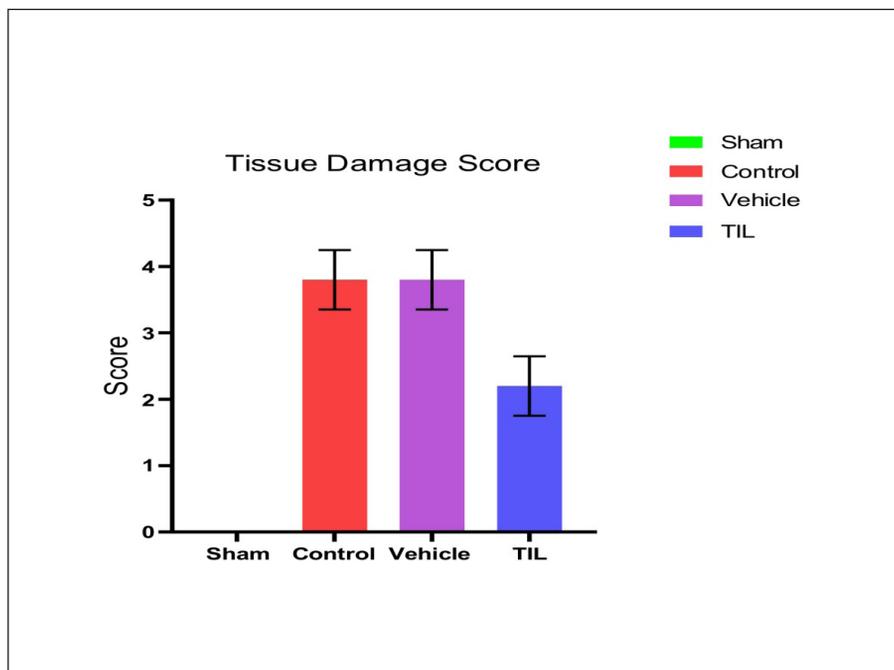


Fig. 7. Severity score mean of histopathological kidney tissue of the 4 groups after collecting all samples. The data are presented as mean \pm SEM, with a sample size of five. For statistical analysis, a one-way ANOVA with Tukey multiple comparisons was used. Control & Vehicle groups vs. Sham group. p -value ≤ 0.01 ; TIL group vs. Control & Vehicle groups: p -value ≤ 0.01 ; TIL: Tilianin.

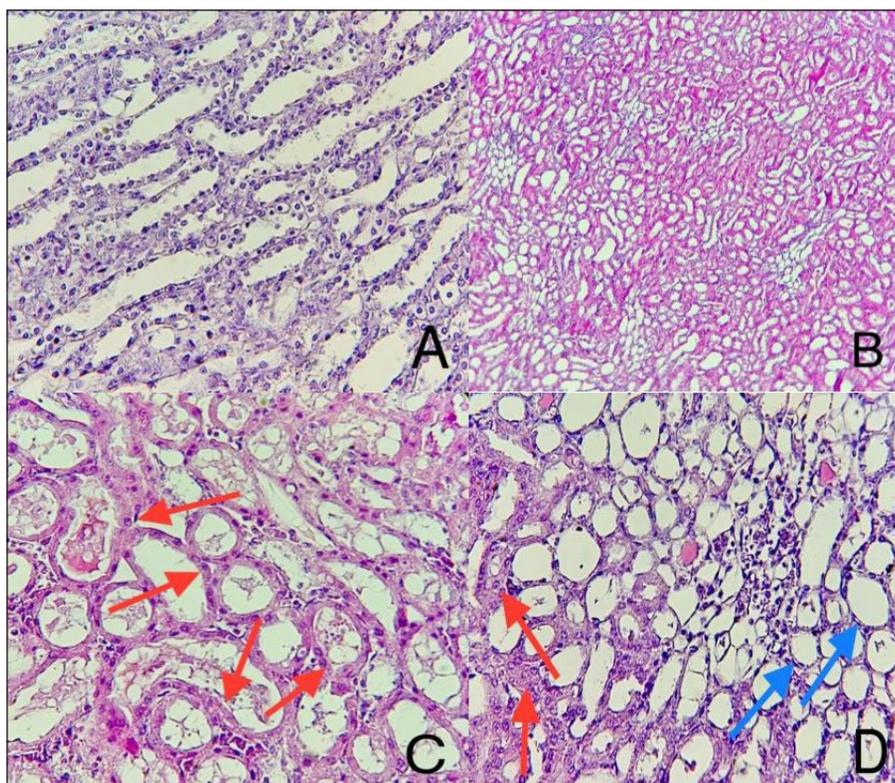


Fig. 8. Renal tissues undergo staining with hematoxylin and eosin. A. Sham group. A cross section of left kidney showed normal histology. 400 \times . B. Control group. Cellular swelling and cytoplasmic eosinophilia, vacuolated epithelium, and Eosinophilic cast. 100 \times . C. Vehicle group cellular swelling and cytoplasmic eosinophilia (red arrow). 400 \times . D. Tilianin treated group. A cross section of left kidney revealed score 2. The renal ischemic changes with damage affecting 35% of renal tubules, including cellular swelling and cytoplasmic eosinophilia (red arrows) and presence of normal renal tubules (blue arrow). 400 \times .

creased cytoplasmic eosinophilia, vascular congestion, and hemorrhage (figure 8C). The kidney tissue cross section of the TIL-treated group showed mild to moderate kidney structural alterations, with damage impacting 35% of the renal tubules (Fig. 8D).

DISCUSSION

The structural and functional alterations take place in response to restoration of blood flow after an ischemia

period is referred as "ischemia reperfusion injury" (IRI). In addition to the negative effects of ischemia, restoration of blood flow can also have harmful impacts on the tissue, such as necrosis of irreparably damaged cells, apparent cell swelling, and uneven restoration of blood flow [1]. Acute kidney injury (AKI) brought on by renal ischemia-reperfusion injury (IRI) is still a significant disorder that is accompanied by an unacceptable high morbidity rate. This leads to extended hospitalization, major renal disease, and occasionally even death [4].

The current experimental study showed that following ischemia reperfusion injury, urea and creatinine serum levels were elevated significantly ($p \leq 0.01$) in both the control and vehicle groups compared to the sham group. These results agreed with other studies. In a rat model research using IRI, blood levels of urea and creatinine were significantly elevated in the control group due to renal tubule injury after a 45-minute ischemia period and a 24-hour reperfusion period [32]. Another study that compared the control group to a sham group likewise found that blood urea and creatinine levels were higher in the control group. This increase in serum nitrogenous waste products, including urea and creatinine, was explained by reduction in oxygen and nutrient availability [33]. Tiliainin pretreated group showed significant ($p \leq 0.01$) reduction in serum urea and creatinine levels as compared with their levels in control and vehicle groups. This research demonstrates that TIL protects parameters of kidney function after development of renal IRI in a rat model (urea and creatinine). This finding agreed with other studies. One trial involving the renoprotective impact of Tiliainin in diabetic rats revealed a significant drop in blood levels of urea and creatinine, which was attributed to the anti-oxidative and anti-inflammatory properties of TIL and led to an improvement in the diabetic rats' renal functioning. The Nrf2/Keap1 signaling pathway is the mechanism via which TIL exerts its antioxidant effects [34]. This experimental study found that the control and vehicle groups' mean severity scores for a portion of the left kidney were significantly ($p \leq 0.01$) higher than the score for the sham group. Cellular edema, cytoplasmic eosinophilia, the disappearance of brush borders, desquamation of epithelial cells, formation of casts in tubular lumens, vascular congestion, interstitial inflammation and bleeding are some of these histological alterations. These results had been consistent with those of other studies. According to one of these investigations, the ischemia reperfusion injury can result in epithelial cell vacuolization, tubule casting, tubule dilatation, and loss of brush border [35]. Other studies have demonstrated renal histological deformation due to ischemia reperfusion damage, including cell desquamation, congestion, necrosis, and apoptosis. Another study found that bilateral IRI could significantly damage the renal tubules, reduce the number of normal renal cells, and increase gene expression that causes macrophage accumulation, which in turn affects cell proliferation, leukocyte migration to the site of injury, suppresses cellular immunity, and ultimately activates the inflammatory response that results in additional renal damage [36]. Tiliainin pretreated group showed a remarkable ($P \leq 0.01$) reduction in renal damage severity compared

to both control and vehicle groups. The score severity mean for the vehicle and control groups indicated severe kidney damage, but the score severity mean for the group receiving Tiliainin treatment suggested mild to moderate damage. Our findings in settlement with other studies. One experimental investigation that evaluated Tiliainin renoprotective impact after giving it to diabetic rats, showed improvement in glomerular and tubular damage. The fact that TIL improved the renal functions of diabetic rats in the results shows that it has anti-oxidative and anti-inflammatory properties [34]. A different experimental study suggested that Tiliainin enhances kidney function following the generation of ischemia-reperfusion damage causes AKI in mice. The tubular damage score dropped to around the half of what was seen in the IRI group after TIL injection, indicating that Tiliainin protects kidney function in vivo by suppressing the ERK/EGR1 signalling pathway [18]. This study discovered that after ischemia reperfusion damage, both the control and vehicle groups' levels of TNF- α and IL-1 β were considerably ($p \leq 0.01$) higher than those of the sham group. The findings supported those of other studies. According to the study, renal ischemia for 45 minutes followed by reperfusion for 6 or 24 hours induced structural and functional damage to the kidneys, as well as systemic and renal inflammation, which is shown by a rise in TNF- α levels in injured tissue [37]. As for IL-1 β , several studies revealed a significant elevation in its level in both control and vehicle groups as compared to sham group.

The development of renal IRI is significantly influenced by the inflammatory response. During the inflammatory process, there is a marked accumulation of neutrophils, and inflammatory factors, chemokines, and adhesion molecules produced as a result of the inflammatory response can further activate neutrophils. Chemotaxis then causes them to infiltrate and aggregate, which exacerbates renal injury [38]. Tiliainin pretreated group showed a remarkable ($p \leq 0.01$) reduction in the level of inflammatory mediators (TNF α , and IL-1 β) in kidney ischemic tissues as compared with the levels of those inflammatory cytokines in both control and vehicle groups. This result indicates that Tiliainin has an anti-inflammatory effect on kidney tissues that underwent ischemia and then had their blood flow restored. Our findings agreed with other experimental studies. According to one study, Tiliainin significantly reduced of the gene expression of TNF- α , suggesting that the main cause of its anti-inflammatory actions was the downregulation of the TNF- α /NF-KB pathways [39]. According to a study, pretreatment with Tiliainin dramatically reduced the production of the pro-inflammatory mediators IL-1 β and TNF- α in a dose-dependent way.

These findings suggested that Tilianin might reduce inflammation. Additionally, this research showed that Tilianin has an anti-inflammatory effect via inhibiting the MAPK/ERK signaling pathway [40]. The current experimental study found that after IRI, that the levels of total antioxidant capacity (TAC) in renal tissues in both the control and vehicle groups significantly decreased ($p \leq 0.01$) compared to the sham group. In comparison to normal tissues, injured kidney tissues (control and vehicle groups) exhibit this downregulation that is explained by an increase in oxidative stress and ROS (sham group). Other studies have endorsed our conclusion. According to one study, reoxygenation of the ischemic kidney causes the creation of ROS, which in turn causes the activation of cytokines and chemokines, which is what causes the large fall in TAC tissue level after IRI. After IRI, the antioxidant defense system deteriorates, and the amount of MDA, a byproduct of lipid peroxidation, increases while antioxidant levels decrease. All of these processes result in cell apoptosis [41]. In comparison to the control and vehicle groups, the Tilianin pretreatment group had a significant ($p \leq 0.01$) increase of TAC expression while a reduction in oxidative stress and the production of free radicals in injured kidney tissues. This suggests that Tilianin has an anti-oxidative effect on damaged renal tissues brought on by ischemia and reperfusion. The effect of Tilianin on total antioxidant capacity in rat or mouse models has not been studied previously. One study, however, examined the cardio-protective action of TIL in a rat model of cardiac ischemia and reperfusion injury and examined the anti-oxidative effect of TIL by assessing malondialdehyde (MDA), an oxidative stress marker, and superoxide dismutase (SOD), an antioxidant enzyme. The outcome confirmed the antioxidant effect of tilianin, showing that it decreased MDA levels and increased SOD activity [20]. The Nrf2 signaling pathway, amplification of Nrf2-regulated genes, and several cytoprotective enzymes, particularly HO-1, are the mechanisms that underlie the enhancement of SOD and the down-regulation of MDA. The activation of the HO-1 enzyme was confirmed to protect cells from oxidative-induced damage [34]. The current experimental investigation showed that following IRI, the levels of caspase-3 in the renal tissues were considerably higher in the control and vehicle groups ($p \leq 0.01$) than in the sham group.

This outcome was consistent with findings from prior research. Caspase-3 levels in the sham group were lower than in the control group, according to an experimental investigation that involved bilateral renal ischemia for 60 minutes, followed by 24 hours of reperfusion in a rat study model [10]. A rat model experiment revealed that after 30 minutes of ischemia and two hours of reperfusion, the levels of caspase-3 in the control and vehicle groups were significantly higher than in the sham group [42]. The ROS generation during IR injury, which also causes the initiation of the inflammatory response and the occurrence of AKI, is responsible for the adverse effects of IR injury on the cell. Furthermore, the generation of ROS activates the signaling pathways that lead to cell death and necrosis [34]. According to the results of the current study, pretreatment with the phenolic flavonoid Tilianin before the induction of ischemia can significantly ($p \leq 0.01$) reduce the expression of caspase-3 in injured kidney tissues as compared to the control and vehicle groups. Our result is in settlement with other studies. In one study, the effectiveness of Tilianin against ischemia injury in neuronal cells was demonstrated. TIL administration reduced pro-apoptotic cytochrome c production and caspase-3 activation in vitro. These findings showed that Tilianin effectively controlled mitochondrial malfunction and played a significant role in preventing apoptosis [21]. When used as a pretreatment for oxidative stress and apoptosis in a cellular model of Parkinson's disease, a study indicated that Tilianin had a dose-dependent reduction in the number of apoptotic cells. Additionally, after receiving Tilianin administration, caspase-3 protein expression levels decreased [40].

CONCLUSIONS

Our results show that Tilianin has a significant nephro-protective effect in renal ischemia-reperfusion injury, as demonstrated by improvements in kidney function parameters (urea and creatinine), and a reduction in the expression of inflammatory mediators (TNF- α , and IL-1 β), a confirmation of the anti-inflammatory effect, an increase in the TAC of cells against free radicals and ROS in ischemic kidney tissues, an indication of antioxidant activity, and a decrease in the level of the anti-apoptotic impact.

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Conflict of interest:

The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

QUANTITATIVE MORPHOLOGICAL ASPECTS OF THE STUDY OF THE FEATURES OF THE REMODELING OF THE HEMOMICROCIRCULATORY CHANNEL OF THE TESTICLES UNDER THE ACTION OF ALUMINUM CHLORIDE ON THE BODY

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ABSTRACT

The aim: To conduct a morphometric analysis of the features of the remodeling of vessels of the hemomicrocirculatory channel of the testicles under the action of aluminum chloride on the body.

Materials and methods: The testicles of 52 white male Wistar rats were morphologically studied, which were divided into two groups: intact and animals injected with aluminum chloride at a dose of 100 mg/kg.

Results: The long-term effect of aluminum chloride on the body led to pronounced changes in the quantitative morphological indicators of the vessels of the hemomicrocirculatory channel of the testes compared to the control ones. The diameter of arterioles of the left testicle decreased by 18.4%, precapillary arterioles by 19.1%, hemocapillaries by 10.1%. The diameter of the postcapillary venules of the left testicle increased by 26.3%, the venules by 26.4%, and the density of microvessels decreased by 30.0%, which indicated the deterioration of its blood supply. The degree of remodeling of microvessels in the right testis was less pronounced. The diameter of arterioles of the right testicle decreased by 17.1%, precapillary arterioles by 18.4%, and hemocapillaries by 9.1%. The diameter of the postcapillary venules of the right testicle increased by 25.5%, the venules by 27.0%, and the density of microvessels decreased by 25.8%.

Conclusions: Long-term action (during the month) of aluminum chloride on the body leads to pronounced remodeling of the vessels of the hemomicrocirculatory channel of the testicles, which is characterized by a pronounced narrowing of its supply (arterioles, precapillary arterioles), exchange (hemocapillaries) links.

KEY WORDS: morphometry, testicles, hemomicrocirculatory channel, aluminum chloride

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INTRODUCTION

Today, there is an increase in the artificial load on the environment, because of which the amount of chemicals and their metabolites, which can negatively affect the organs and systems of the body and worsen the course of various pathologies, is increasing. It is known that aluminum and its compounds have a toxic effect on living organisms due to their accumulation in organs and tissues, which is accompanied by a violation of normal metabolic processes and the development of pathology. Inorganic metal salts, which include aluminum chloride, are particularly harmful to living organisms [1].

This substance is widely used by man in various spheres of his activity: oil, as a catalyst, cosmetics industry for the production of antiperspirants and deodorants, and medicine. The reproductive system in men

is very sensitive to the influence of exogenous factors and can be complicated by azoospermia (absence of spermatozoa in the ejaculate) [2, 3]. In recent decades, indicators of men's reproductive and sexual health have been decreasing in many countries of the world, but in Ukraine, they have a rapid and extremely negative trend [3]. Modern researchers emphasize the underestimation of the negative impact of environmental factors on the generative function in men.

It is also known that functional and structural changes in the vessels of the hemomicrocirculatory channel are the first early signs of the effects of negative factors of endogenous and exogenous origin on the body [4], which clearly occur under the conditions of increased formation of reactive oxygen species with a concomitant deficiency of antioxidant protection [5-8]. Against the background of age-related pathobiochemical

changes, asthenodepressive syndrome, memory and sleep disorders develop, and efficiency is reduced. Age-related androgen deficiency is manifested not only by sexual dysfunction, but also associated with age-related diseases (obesity, diabetes, coronary heart disease, osteoporosis, etc.), which exacerbate dysfunction of the male reproductive system [9-11].

Thus, in the process of spermatogenesis an important place belongs to the interaction of many factors, first – hormonal, as well as nervous, immune, genetic, which are based on subtle, still little studied molecular mechanisms of regulation. The vessels of the hemomicrocirculatory channel, where transcapillary exchange takes place, play an important role in the trophic supply of cells and tissues and in the pathomorphogenesis of their injuries. It should be noted that the features of the remodeling of the hemomicrocirculatory channel of the testes during the action of aluminum chloride on the body have not been sufficiently studied.

THE AIM

To carry out a morphometric analysis of the features of the remodeling of vessels of the hemomicrocirculatory channel of the testicles under the action of aluminum chloride on the body.

MATERIALS AND METHODS

The testes of 52 sexually mature white male Wistar rats, which were divided into two groups, were studied using a complex of morphological methods. The 1st group (intact) included 26 animals that were in normal vivarium conditions, the 2nd – 26 rats that were injected intraperitoneally with aluminum chloride at a dose of 100 mg/kg [12]. The experimental animals were euthanized one month after the start of the experiment.

The hemomicrocirculatory channel of the testes was studied by filling their vessels with a carcass-gelatin mixture, which was injected through the abdominal aorta. 3-4 hours after filling the bloodstream of the testicles with the indicated mixture, they were collected and fixed in a 10% neutral formalin solution for 2 weeks. Sections with a thickness of 30-40 μm were made on a freezing microtome, which were dehydrated in ethyl alcohol, clarified in methyl ether of salicylic acid and placed in polystyrene. Examination of micro-preparations was performed on a Nikon Eclipse CI-E microscope. Microscopy of microscopic images was performed using a Sigeta M3CMOS 14000 camcorder and Toup View software on a personal computer. [13]. The diameters of arterioles (DA), precapillary arterioles (PA), hemocapillaries, postcapillary venules (PV),

venules (V) and microvessel density (MD) per 1 mm^2 of the tissues of the left (LT) and right testicles (RT) were determined morphometrically [4]. Quantitative indicators were processed statistically. The processing of the obtained results was carried out in the department of systematic statistical research of I. Horbachevsky Ternopil National Medical University the STATISTIKA software package. The difference between the compared values was determined by the Student and Mann-Whitney test. Conducting experiments and euthanasia of experimental animals was carried out in compliance with the "General Ethical Principles of Animal Experiments" adopted by the First National Congress on Bioethics (Kyiv, 2001), in accordance with the "European Convention for the Protection of Vertebrate Animals Used for Research and Other Scientific Purposes" as well as the Law of Ukraine "On the Protection of Animals from Cruelty" (dated February 21, 2006).

RESULTS

A comprehensive analysis of the data (Table I) established that long-term exposure to aluminum chloride led to pronounced changes in the quantitative morphological indicators of blood vessels of the hemomicrocirculatory channel compared to control ones. Thus, the diameter of the arterioles of the left testicle in simulated experimental conditions with a pronounced statistically significant difference ($p < 0.001$) decreased from $(18.20 \pm 0.30) \mu\text{m}$ to $(14.85 \pm 0.24) \mu\text{m}$, by 18.4%.

The diameter of the precapillary arterioles of the specified testicle also changed similarly. Thus, the diameter of the precapillary arterioles of the intact left testicle was equal to $(10.82 \pm 0.12) \mu\text{m}$, and in the 2nd group of observations - $(8.75 \pm 0.12) \mu\text{m}$. The given morphometric parameters were statistically significantly ($p < 0.001$) different from each other. At the same time, the last quantitative morphological indicator was lower than the similar control by 19.1%.

The lumen of the hemocapillaries of the left testicle in the studied conditions of the experiment also decreased by 10.1% with a high degree of statistically significant difference ($p < 0.001$).

Quantitative morphological analysis established that the venous vessels of the hemomicrocirculatory channel of the left testicle expanded when aluminum chloride was applied to the body of experimental animals. Thus, in the simulated experimental conditions, the diameter of the post-capillary venules of the left testicle of the animals increased statistically significantly ($p < 0.001$) by 26.3%, and the venules increased by 26.4% ($p < 0.001$). At the same time, the density of microvessels per unit tissue area of the examined organ significantly

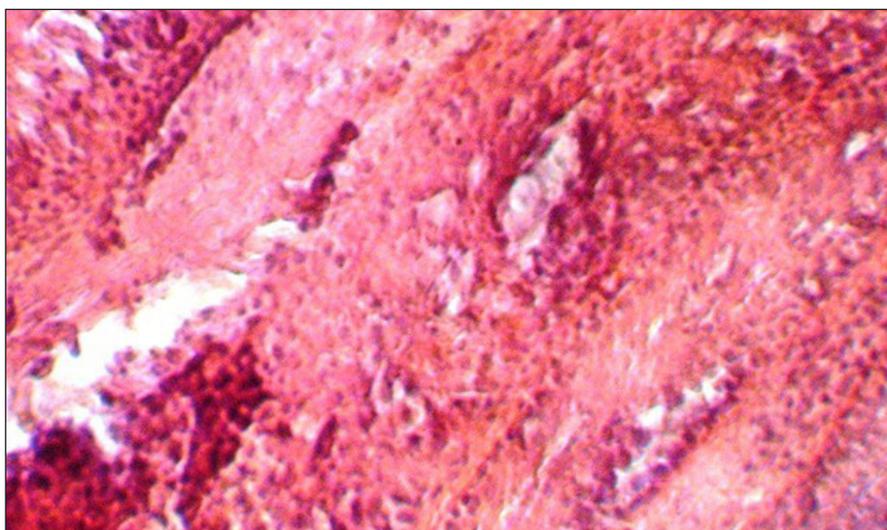


Fig. 1. Destructive processes in the vessel wall desquamation of endotheliocytes, narrowing of the lumen of the vessels of a laboratory sexually mature white male rat under the action of aluminum chloride on the body.

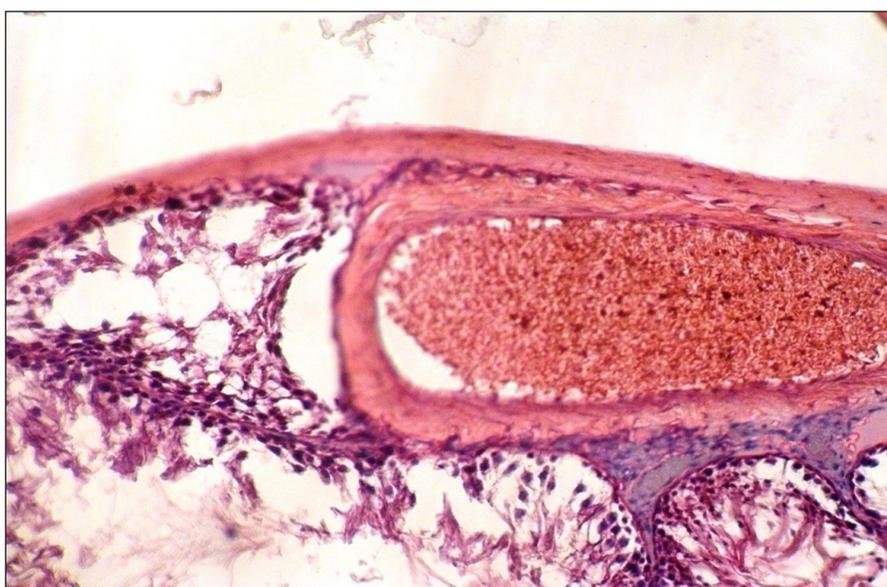


Fig. 2. Full-blooded vein, destructive processes in its wall, desquamation of spermatogenic epithelium in tortuous seminiferous tubules

decreased by almost 30.0% ($p < 0.001$), which indicated the deterioration of its blood supply [2, 4].

The structural rearrangement of the vessels of the hemomicrocirculatory channel of the right testicle during the action of aluminum chloride on the body of experimental animals turned out to be similar to the one described above. It is worth noting that the analysis of the investigated morphometric parameters established that the degree of remodeling of the microvessels of the hemomicrocirculatory channel in the left and right testicles turned out to be different. Thus, the diameter of the arterioles of the right testicle in the simulated conditions of the experiment with a pronounced statistically significant difference ($p < 0.001$) decreased by 17.1% compared to the similar control indicator, the diameter of the precapillary arterioles – by 18.4% ($p < 0.001$), and hemocapillaries - by 9.1% ($p < 0.001$).

Venous vessels of the hemomicrocirculatory channel (capillary venules and venules) expanded under the

influence of aluminum chloride. Thus, the diameter of the postcapillary venules of the right testicle increased by 25.5%, and the diameter of the venules increased by 27.0% ($p < 0.001$) with a high degree of statistically significant difference ($p < 0.001$). The density of microvessels under these experimental conditions decreased from (3836.8 ± 30.3) to (2846.9 ± 22.1) , so it is by 25.8% ($p < 0.001$).

DISCUSSION

Man-made loads with aluminum compounds are accompanied by hemodynamic disorders, which are manifested to one degree or another by pronounced testicular ischemia. [1, 6, 7]. The cells of the spermatogenic epithelium show the greatest sensitivity to hypoxia. According to most authors [1, 3, 6, 7] violations of spermatogenesis are directly dependent on circulatory disorders and are irreversible.

Table I. Morphometric characteristics of the hemomicrocirculatory channel of the test animals (M±m)

Indicator	Group of animals	
	1st group (intact)	2nd group
	Left testicle	
diameters of arterioles, μm	18.20±0.30	14.85±0.24***
precapillary arterioles, μm	10.82±0.12	8.75±0.12***
hemocapillaries, μm	6.12±0.09	5.50±0.09***
postcapillary venules, μm	12.58±0.15	15.90±0.15***
venules, μm	26.57±0.30	33.60±0.30***
microvessel density	3843.3±28.2	2690.5±21.3***
	Right testicle	
diameters of arterioles, μm	18.22±0.30	15.10±0.27***
precapillary arterioles, μm	10.85±0.12	8.85±0.09***
hemocapillaries, μm	6.12±0.09	5.56±0.06***
postcapillary venules, μm	12.54±0.15	15.75±0.12***
venules, μm	26.53±0.30	33.70±0.27***
microvessel density	3836.8±30.3	2846.9±23.1***

***-p<0.001

The predominance of expansion of venous microvessels of the hemomicrocirculatory channel, venous full blood was complicated by hypoxia, in which oxygen transport to cells is disrupted, energy synthesis decreases intracellular ATP decreases, functional-metabolic and trophic disturbances occur, which significantly affects the vital activity of testicular structures. The latter is confirmed by the presence of dystrophy, necrobiosis of cells and tissues of the examined organ, infiltrative and sclerotic processes [1, 6].

The leading in of aluminum chloride into the body of sexually mature male laboratory rats leads to complex general biological processes that arise and develop in the organs and systems of the body during its adaptation to a new level of life [6, 9]. It should be noted that detailed and objective knowledge of compensatory-adaptive processes in cotyledons needs its own solution.

The obtained morphometric indicators of the studied vessels indicate that the influence of aluminum chloride leads to a pronounced structural rearrangement of the vessels of the hemomicrocirculatory channel of the testes. At the same time, it should also be noted that the microvessels of the supply and exchange links are narrowed, and those of the outlet part of the hemomicrocirculatory channel are significantly expanded. Pronounced expansion of post-capillary venules and venules led to venous congestion and stasis, which cause edema, plasmorrhagia in the vascular walls and perivascular stroma, which supported and intensified the state of hypoxia and deterioration of cell and tissue trophism. The latter contributed to the increase in ede-

ma and were complicated by dystrophic, necrobiotic changes in cells and tissues. Damage to endotheliocytes led to a decrease in the production of nitric oxide, an increase in the synthesis of endothelin-1, which increased vasospasm. The specified hemomicrocirculatory changes were accompanied by deterioration of blood rheology, increased permeability of the walls of microvessels, plasmorrhagia not only of their walls, but also of the paravascular stroma with blood proteins. At the same time, perivascular swelling, saturation of the surrounding stroma with proteins and blood-forming elements, hypoxia, defibrillation, disorganization and dissociation of fibrous structures, significant deterioration of the diffusion of nutrients and oxygen, which was complicated by dystrophic and necrobiotic changes of stromal structures, vascular endotheliocytes and spermatogenic epithelium, fibroplastic activity, polymerization and saturation of collagen fibrils with glycosaminoglycans [4, 6, 9].

During histological examination of micro-preparations, an increase in the lumens of mainly venous vessels of the testicles, especially post-capillary venules and venules, which are dilated, filled with blood, with foci of stasis, thrombosis, diapedesis, paravascular hemorrhages, was revealed. Hemocapillaries are mostly narrowed, spasmodic. The wall of arterioles is thickened, their lumen is narrowed, some endotheliocytes are dystrophically and necrobiotically altered and desquamated. In individual arterioles, the following were observed: destruction of membranes, structural changes of myocytes, phenomena of myoelastofibrosis, which are presented in Fig 1.

In the lumen of the venules, formed elements of the blood, mainly erythrocytes, were found, the shape of which was elongated oval. Edema was observed in the cytoplasm of endotheliocytes. Cytolemma of endotheliocytes of uneven thickness and contours. This structure formed protrusions of various shapes and sizes, as well as microvilli in the lumen of microvessels. The cytolemma of endotheliocytes also formed recesses in their cytoplasm.

Pronounced venous congestion was complicated by hypoxia, which led to dystrophic and necrobiotic changes in endotheliocytes, vascular myocytes, spermatogenic epitheliocytes, stromal structures, infiltration and sclerosis (Fig 2).

The contours of the walls of venous vessels are unclear, thickened. Swollen and sclerosed cells alternate, their contours are tortuous, twisted, the lumens are deformed. Endotheliocytes of venous vessels are increased in size with the phenomena of edema, dys-

trophy, and necrobiosis. There were cells with desquamation of endotheliocytes, pyknosis and lysis of their nuclei. Structural changes dominated in the left testicle, which is associated with the peculiarities of the venous outflow from the indicated organ [3, 4].

CONCLUSIONS

Long-term action (during the month) of aluminum chloride on the body leads to pronounced remodeling of the blood vessels of the hemomicrocirculatory channel of the testes, which is characterized by a pronounced narrowing of its supply (arterioles, precapillary arterioles), exchange (hemocapillaries) links and expansion of postcapillary venules and venules, venous congestion, hypoxia, dystrophic, necrobiotic changes in cells and tissues, infiltrative and sclerotic processes that dominate the left testicle.

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The Authors declare no conflict of interest.

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ORIGINAL ARTICLE

PECULIARITIES OF PULMONARY VENTILATION RESPONSE TO DOSED HYPOXIA IN ELDERLY PEOPLE WITH IMPAIRED GLUCOSE TOLERANCE

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ABSTRACT

The aim: To determine the peculiarities of the response of pulmonary ventilation to hypoxia in elderly people with impaired glucose tolerance

Materials and methods: Forty-three elderly people were examined, including 20 patients with impaired glucose tolerance and 23 healthy individuals with preserved glucose tolerance. Fasting plasma glucose and insulin concentrations were determined, and the HOMA-IR insulin resistance index was calculated. Under conditions of normoxia and during a dosed hypoxic test (12% oxygen, duration 20 min), blood saturation and lung ventilation parameters were monitored.

Results: Under conditions of normoxia, the indicators of lung ventilation function did not differ between the groups of elderly people with impaired and preserved glucose tolerance. Under conditions of hypoxia, elderly people with impaired glucose tolerance had a less significant increase in ventilation, despite the development of more severe arterial hypoxemia. This leads to a decrease in the ventilatory response to hypoxia in case of impaired carbohydrate metabolism.

Conclusions: In people with impaired glucose tolerance, a less significant ventilatory response to hypoxia is combined with more pronounced insulin resistance.

KEY WORDS: impaired glucose tolerance, aging, hypoxia, ventilation, insulin resistance

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INTRODUCTION

Disorders of glucose homeostasis are an important medical and social problem in the world. This is primarily due to their high prevalence, development of complications and economic losses. Thus, in 2015, there were about 415 million people in the world with disorders of glucose homeostasis (elevated fasting blood glucose level, impaired glucose tolerance), and according to the forecast, their number will increase to 642 million in 2040 [1]. Today, disorders of glucose homeostasis are considered an important risk factor for the development of cardiovascular disease and type 2 diabetes mellitus [2]. Epidemiological studies show that within a year, 3.6% to 8.7% of people with dysglycemia become patients with type 2 diabetes mellitus [3]. This gives grounds to consider impaired glucose homeostasis as an intermediate stage before the development of diabetes, i.e., as prediabetes [4].

Previous studies have shown a significant increase in the prevalence of glucose homeostasis disorders in older age: from 8.8% in men and 11% in middle-aged women to 24.3% in men and 34.7% in women over 85 years of age [5]. This indicates a link between age and carbohydrate metabolism disorders.

The causes of carbohydrate metabolism disorders in aging may include poor nutrition, reduced physical activity, decreased muscle mass, decreased insulin secretion, and the development of insulin resistance [6]. According to researchers, insulin resistance (IR) is the most important cause of impaired carbohydrate tolerance in the elderly [6].

Hypoxia plays an important role in glucose homeostasis disorders and in the development of diabetes complications [7]. Hypoxia controls the activation of HIF-1 α , a hypoxia-inducible transcription factor, and through it VEGF, a vascular endothelial growth factor [8]. In the presence of elevated glucose concentrations, cells and tissues are exposed to elevated levels of glycation end products that inhibit HIF-1 α function [7]. This leads to increased degradation of HIF-1 α under conditions of hypoxia and hyperglycemia [7]. The consequence is a decrease in the adaptive capacity of cells and their survival [7, 8]. Tissue hypoxia also promotes the activation of proinflammatory cytokines, which can cause the onset and progression of carbohydrate tolerance disorders [9].

On the other hand, hypoxia and hypoxic shifts are the cause or characteristic feature of age-related decline in the body's functional capacity and adaptive capacity [10].

With aging, oxygen supply to tissues and oxygen tension in tissues decrease [11]. Studies have also shown that the body's resistance to hypoxia decreases with aging [11, 12].

Among the mechanisms of adaptation to hypoxia, the respiratory system plays a key role, as it responds most quickly to the development of arterial hypoxemia. Under conditions of oxygen deficiency, lung ventilation increases and bronchial patency increases [12].

At the same time, changes in pulmonary ventilation under hypoxia in elderly people with impaired glucose tolerance (IGT) remain unclear.

THE AIM

The aim of our study was to determine the peculiarities of the response of pulmonary ventilation to hypoxia in elderly people with impaired glucose tolerance (IGT).

MATERIALS AND METHODS

The study was conducted in accordance with ethical guidelines. Participation in the study was voluntary, all subjects received detailed information about the study and signed an informed consent. The study procedures, patient information, and the informed consent form were approved by the Ethics Committee of the Clinical Sector of the Dmitry F. Chebotarev Institute of Gerontology of the National Academy of Medical Sciences of Ukraine (Protocol 9 of June 11, 2013).

We examined 20 people aged 60-74 years with IGT, which was diagnosed on the basis of the results of an oral standard glucose tolerance test (OGTT) [2]. The criterion for IGT was a glycemic level of 7,8 to 11,1 mmol*l⁻¹ after 2 hours of OGTT. As a control group, 23 practically healthy people aged 60-74 years with preserved glucose tolerance (PGT) were examined, in whom the plasma glucose level after 2 hours of OGTT was less than 7.8 mmol*l⁻¹.

The index of insulin resistance (HOMA-IR - Homeostasis Model Assessment for Insulin Resistance) was calculated by the formula:

$$\text{HOMA-IR} = \text{fasting plasma glucose} * \text{fasting plasma insulin} / 22,5.$$

The glucose concentration was determined by the glucose oxidase method on a BTS-330 analyzer using Glucose reagents (Bio LATEST Lachema Diagnostica, Germany), and the plasma insulin concentration was determined by an enzyme-linked immunosorbent assay using a DRG Insulin ELISA kit (DRG Instruments GmbH, Germany).

A dosed hypoxic test was performed in a sitting position no earlier than 2 hours after breakfast using a standard certified gas mixture containing 12 % ox-

xygen and 88 % nitrogen. The duration of the test was 20 minutes. During the hypoxic test, blood saturation (SpO₂) was recorded using the "UM-300" monitor (UTAS, Ukraine), respiratory volume (VT) and respiratory rate (F) using the "Hypotron" apparatus (Scientific Research Institute "APRODOS" of National technical university of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Ukraine). SpO₂ and pulmonary ventilation were recorded under normoxia conditions (before the hypoxic test for 5 minutes), then during the hypoxic test and for 5 minutes after the hypoxic test. The degree of SpO₂ decrease during the hypoxic test reflects the body's ability to withstand hypoxic exposure, i.e., characterizes resistance to hypoxia [13]. It is worth noting that inhalation of a hypoxic gas mixture containing 12 % oxygen for 20 minutes is completely safe for humans, as evidenced by the widespread use of hypoxic tests in both healthy and sick people [14].

The obtained data were processed by the methods of variational statistics using the computer program "Statistica 7.0 for Windows". The studied indicators had a distribution close to normal. Average values of indicators (M), their errors (m) were calculated. Differences in the average values of the indicators in the groups were evaluated according to the Student's test. Pearson's correlation analysis was performed. The critical level of statistical significance was 0.05.

RESULTS

Under conditions of normoxia, the indicators of lung ventilatory function did not differ between groups of elderly people with IGT and PGT (table I).

In response to dosed hypoxia, ventilation rates increased in both patients with PGT and patients with IGT (table I).

At the same time, certain differences in the response of pulmonary ventilation to dosed hypoxia in elderly people with PGT and those with IGT were found. In subjects with IGT under hypoxia, the increase in VT and, accordingly, VE was less than in subjects with PGT, despite the development of more severe arterial hypoxemia. As a result, under hypoxia, the ratio $\Delta\text{VE}/\Delta\text{SpO}_2$, which characterizes the ventilatory response to hypoxia, decreased to a lesser extent in patients with IGT than in patients with PGT (table I).

The insufficient ventilatory response to hypoxia in elderly people with IGT is likely to be one of the factors in reducing their resistance to hypoxia.

In the study of the relationship between ventilation response to hypoxia and the insulin resistance index, the following was found. In patients with IGT, changes in minute ventilatory volume (ΔVE) during hypoxia

Table I. Indicators of ventilation function of lungs in elderly people with IGT and PGT, $M \pm m$

Indicator	Group with PGT	Group with IGT
VT in normoxia, l	0,61 ± 0,02	0,59 ± 0,03
VT in hypoxia, l	0,69 ± 0,03	0,63 ± 0,03
ΔVT, l	0,08 ± 0,007	0,04 ± 0,007*
F in normoxia, min-1	13,10 ± 0,23	13,01 ± 0,25
F in hypoxia, min-1	14,32 ± 0,15	14,71 ± 0,15
ΔF, min-1	1,22 ± 0,09	1,70 ± 0,07
VE in normoxia, l*min-1	7,99 ± 0,26	7,68 ± 0,22
VE in hypoxia, l*min-1	9,88 ± 0,28	9,27 ± 0,30
ΔVE, l*min-1	1,89 ± 0,08	1,59 ± 0,06*
SpO2 in normoxia, %	95,74±0,15	95,50 ± 0,17
SpO2 in hypoxia, %	80,74 ± 0,16	78,13 ± 0,14*
ΔSpO2, %	-15,00 ± 0,14	-17,37 ± 0,11*
ΔVE/ΔSpO2	-0,126 ± 0,003	-0,092 ± 0,005*

Notes: all shifts are significant, $p < 0.05$; * - differences are significant compared with the indicators of elderly people with VT, $p < 0.05$; VT - respiratory volume, F - respiratory rate, VE - minute respiratory volume, Δ - shift in hypoxia

correlated with the HOMA-IR index of insulin resistance ($r = -0.37$, $p = 0.0012$). The regression analysis showed an inverse linear relationship between these parameters.

The analysis also revealed an inverse correlation ($r = -0.36$, $p = 0.0019$) between VE shifts during hypoxia, on the one hand, and changes in glucose levels after 2 hours of OGTT, on the other hand, in patients with IGT.

DISCUSSION

The question arises: why do elderly people with IGT have a reduced ventilatory response to hypoxia? It is known that up to 70% of circulating blood glucose is consumed by the brain [15]. Our previous studies have shown that hypoxia reduces blood glucose levels, especially in people with IGT [16]. In order to compensate for the energy supply of the brain under hypoxia, glucose transport to the brain is activated. Thus, the level of the insulin-independent glucose transporter GLUT-3 increases [17]. At the same time, it is known that in IR, cells lose the ability to respond to the metabolic activity of insulin and insulin-mediated processes in the brain, in particular in the hypothalamus, hippocampus and cerebral cortex [18]. Under conditions of oxygen deficiency, despite increased glucose transport, defective insulin signaling leads to a shortage of energy substrates in brain neurons. In IR, the importance of activation of free radical processes in the brain during hypoxia also increases. This can contribute to an excessive increase in the level of membrane lysophospholipids, which

leads to a violation of the functional activity of mitochondria and a decrease in the synthesis of macroergic compounds [19].

The described processes reduce the energy supply of the brain and form a different pattern of the activity of the neurons of the respiratory center. This can lead to a delayed and insufficient response to hypoxia of the respiratory center neurons in elderly people with IGT.

On the other hand, the mechanisms of ventilation regulation, its response to hypoxia, and carbohydrate metabolism may interact [20]. Therefore, it is possible that functional insufficiency of ventilation regulation contributes to carbohydrate metabolism disorders. In turn, disorders of carbohydrate metabolism, which, in particular, develop in elderly people with IGT, negatively affect metabolism and can cause changes in the sensitivity of central and peripheral chemoreceptors. This leads to a decrease in the sensitivity of the chemoreflex mechanism of compensation for arterial hypoxemia. As a result of these complex mechanisms, the respiratory center's response to hypoxia in elderly people with IGT is reduced.

RESPIRATORY RESPONSE TO HYPOXIA AND INSULIN RESISTANCE

It is known that a constant oxygen tension in tissues is essential for optimal cellular metabolism. The activation of ventilation under the influence of hypoxic stress is aimed at compensating for oxygen deficiency to meet

the body's metabolic needs. The activity of metabolic processes, in particular, carbohydrate metabolism under hypoxia, directly depends on the adequacy of the body's compensatory reactions. It is hypoxia, that is, the inability to meet the oxygen needs of the body, that leads to the development of pathological changes in carbohydrate metabolism. However, the relationship between changes in pulmonary ventilation to hypoxia and insulin resistance in elderly people with IGT is still unclear.

These data suggest the existence of a cause-and-effect relationship between IGT and insufficient response of lung ventilation to hypoxia in the elderly. The identified dependencies do not allow us to determine what is the cause and what is the effect. However, they show an

important role of lung ventilation in the development of adaptive reactions to hypoxia in elderly people with IGT.

CONCLUSIONS

1. Ventilatory function of the lungs under condition of normoxia does not differ in elderly people with preserved and impaired glucose tolerance.
2. In elderly people with impaired glucose tolerance, the ventilator response to dosed hypoxia is less than in healthy people with preserved glucose tolerance.
3. In elderly people with elevated glycemia and insulin resistance, the ventilator response to hypoxia decreases, which leads to a limitation of the body's adaptive capacity in hypoxic conditions.

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ACTIVITY-BASED COSTING: A PRACTICAL MODEL OF COST CALCULATION IN PSYCHIATRIC HOSPITALS

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ABSTRACT

The aim: This paper is an analytical and descriptive study of the use of the Activity Based Costing (ABC) system in mental healthcare institutions for the allocation of overhead costs of auxiliary units that provide non-medical services. The realistic estimates obtained using this costing system will help improve the allocation of resources, especially in the conditions of increased number of patients, and to control overhead costs, ensuring the reduction of non-productive expenses, increasing the efficiency and effectiveness of the mental health and psychosocial support institution.

Materials and methods: The study describes and uses ABC as a system and method for calculating inpatient care costs in 2022. The cost of medical services will be calculated as the sum of direct and overhead costs. Overhead costs into inpatient care consist of medical support units overhead costs and non-medical support units overhead costs. Overhead costs in non-medical support units were allocated to the cost of services within the cost centers: hospital departments that specialize in the treatment of specific mental health conditions. Expert opinion regarding the alignment of the cost allocation base with the factors identified for the allocation of each group of overhead costs in the mental health and psychosocial support facility was taken into account.

Results: Calculations showed the impracticality of using a single tariff for psychiatric care medical services for adults and children in inpatient conditions, since there are cost centers, namely, clinical departments of health care institutions, which consume a larger amount of resources. In particular, these are the departments providing the treatment of psychotic disorders caused by taking psychoactive substances, as well as schizophrenic, organic disorders, including symptomatic, mental disorders). Accordingly, the tariff for such medical services should be higher.

Conclusions: ABC is applicable in mental health care institutions and can help to efficiently cost processes, and thereby overcome a key challenge: to overestimation or underestimation of costs into a medical services. Cost assignment through ABC generated more accurate cost estimates, which suggests that model of cost calculation ABC is more able to address complexity in mental health care institutions.

ABC should be gradually incorporated into an estimated approach to costing leads, to progressively bring reliable cost-accounting practices to mental health care institutions

KEY WORDS: activity-based costing, inpatient care costs, cost centers, cost driver, cost management system of mental health care in Ukraine

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INTRODUCTION

The war in Ukraine has largely contributed to the intensive growth of the number of people who will likely have problems with mental health. According to global estimates, the experience of military conflict is the cause of mental health problems in 20% of the population [1]. A sociological research shows that in the conditions of the active phase of the military conflict, at least 50% of the population of Ukraine experiences a potentially traumatic experience [2]. Internally displaced persons, military personnel, veterans and their family members as well as those who have experienced the loss of a family member as a result of hostilities constitute the largest share. The most common mental problems and

disorders currently recorded in Ukraine include long-term stress, depressive disorder, anxiety disorder, and post-traumatic stress disorder [3-5].

Despite the significant increase in the number of people in need of mental health care, the functioning of mental health and psychosocial support institutions in Ukraine and their funding have remained the same. In particular, despite the transition to the Strategic Procurement Concept [6] according to which the tariff for medical services has been formed, mental health care and psychosocial support institutions are still under estimated funding. That is, institutions develop an approximate plan of needs based on the expenses of the previous year, adjust them according to the changes in prices and volumes of activ-

ity, without any comprehensive analysis of the activities of their institution. Therefore, the funds are not always used efficiently and are often insufficient to cover the expenses of the reporting period. This process, despite its time-consuming nature, does not provide any opportunity to clearly calculate the cost of medical services received by patients and to manage the funds of health care institutions, avoiding unproductive costs.

The dominant factor in avoiding unproductive expenses, in particular, when calculating costs in mental health care institutions, is the use of innovative approaches to the formation of management systems. These approaches are aimed not only at the additional attraction of budget funding in connection with the increase in the number of services provided, but also at the creation of new cost-optimizing approaches to management.

The cost management system of mental health care and psychosocial support institutions in Ukraine in the face of the growing number of patients is of decisive importance. It should be aimed at the correct and accurate calculation of the cost of the provided service. Due to the specificity of mixed funding of health care facilities, it is impossible to calculate the cost of both the medical service and the finished case of patient treatment.

The use of an estimated approach to costing leads to overestimation or underestimation of costs due to the inability to reconcile the bases of cost distribution with the factors that determine them. Activity Based Costing (ABC) is a costing system that helps calculate the actual costs associated with the provision of health services and connect them to the resources they consume [8-9]. It also contributes to determining priorities in cost management, providing more detailed information about activity costs, which, as a rule, leads to the elimination of unproductive costs.

THE AIM

Accurate calculation of the cost of services is very important for reducing expenses. Thus, using the potential of the ABC system, this study aims at calculating the costs of responsibility centers for inpatient care in the allocation of overhead costs of auxiliary units that provide non-medical services, using the example of the «Regional Clinical Psychiatric Hospital No. 3» Communal Non-Profit Enterprise of the Kharkiv Regional Council (hereinafter - «Regional Clinical Psychiatric Hospital No. 3» KRC CNE).

MATERIALS AND METHODS

This study is an analytical-descriptive research conducted through studying of open-access financial documents of “Regional Clinical Psychiatric Hospital

No. 3” KRC CNE.

The study of the cost of services was conducted using the ABC method based on financial information for 2022. The ABC estimates costs using activities as the basis for calculations.

The steps of data analysis (using ABC) in this study divided into three major: (1) using the primary data to determination of the types of Activities of the Hospital’s Responsibility Centers; (2) classifying of the Activity of Responsibility Centers; (3) Identifying the overhead costs into inpatient care, which accumulated in non-medical units and the allocation of overhead costs of non-medical units to the medical services.

RESULTS

Calculation according to ABC costing was carried out in several stages described below.

1. DETERMINATION OF THE TYPES OF ACTIVITIES OF THE HOSPITAL’S RESPONSIBILITY CENTERS

The ABC process begins with the development of a map of the activities of the mental health and psychosocial support institution and the division of provided services into:

- basic medical and diagnostic services directly related to the diagnostic and treatment process;
- non-medical services: public, administrative, and support services that ensure the functioning of the hospital, but are not related to the main services to patients.

The list of the main services provided by the core units of the health care institution was determined in accordance with the Program of Medical Guarantees (PMG), «Inpatient Psychiatric Care». Based on the classification of core and non-core units, one can distinguish the main responsibility centers that perform certain types of medical activities. It simplifies the process of allocating overhead costs accumulated by non-core units of the hospital.

On the example of the “Regional Clinical Psychiatric Hospital No. 3” KRC CNE, we identified three units of the functioning of the mental health care and psychosocial support institution. Within the scope of meeting the need for obtaining basic services, the following are highlighted (Fig. 2):

(1) major cost centers: hospital departments that specialize in the treatment of specific mental health conditions; (2) auxiliary medical units that offer additional services for the diagnosis, treatment, and prevention of mental health disorders and provide for

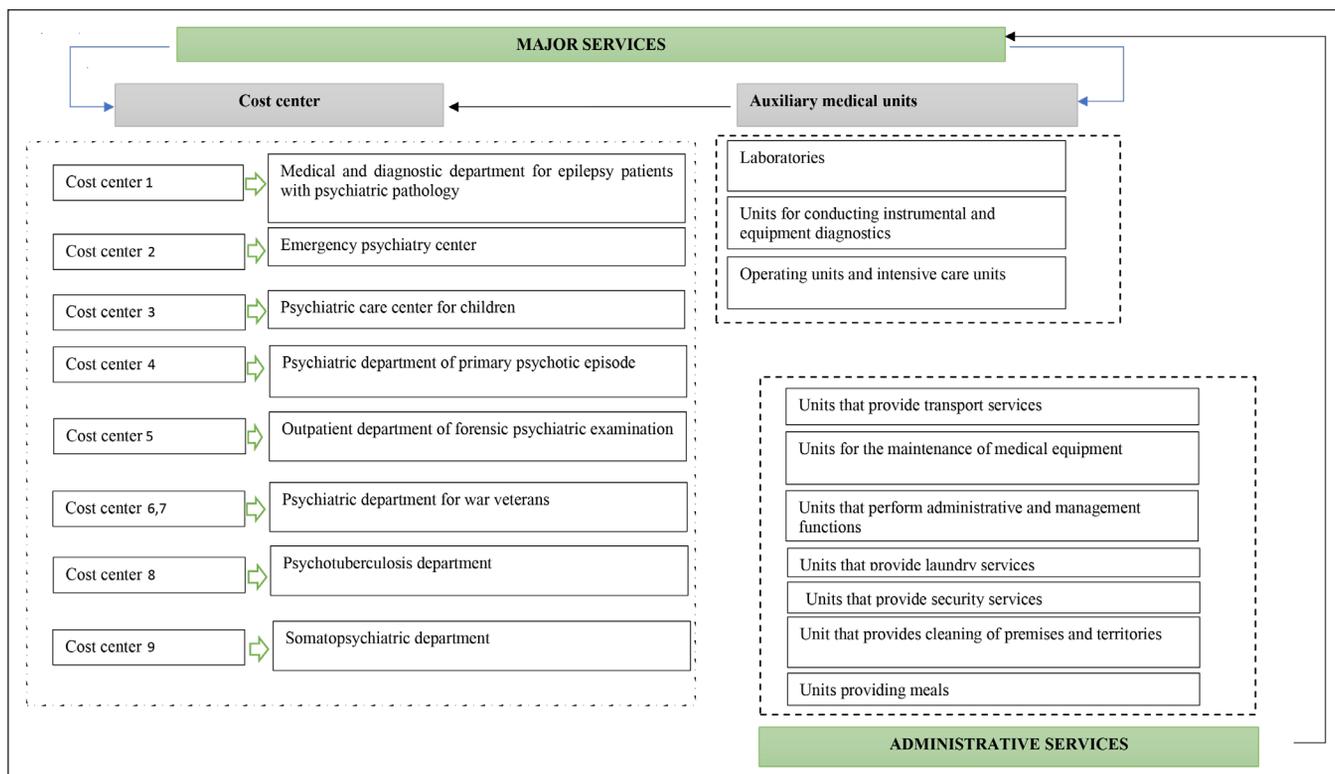


Fig.1. Defining Activity Centers in «Regional Clinical Psychiatric Hospital No. 3» KRC CNE.

the activities of responsibility centers; (3) auxiliary units that offer non-medical services, namely, administrative and maintenance services.

2. ANALYSIS OF THE ACTIVITY OF RESPONSIBILITY CENTERS

At this stage, all costs accumulated by the cost center of basic services are divided into direct costs, which are directly related to the cost of the medical service, and overhead costs.

Direct costs that can be obtained from the hospital's data systems should include only the labor costs of the treating physician and the cost of medicine if it is included in the Medical Guarantee Program.

All other costs incurred by the healthcare facility will be overhead costs and therefore need to be allocated using a cost driver (allocation factor) to be included in the cost of the healthcare service.

In accordance with the cost items, the cost driver is calculated for each item of indirect costs for each responsibility center. A cost driver is a numerical multiplier that characterizes the proportion of the allocation of certain costs to one or another responsibility center. Cost drivers determine the cost of each service in the responsibility center.

The allocation of medical overhead costs within the main clinical departments can be performed by the

direct allocation method (VBC method), where the cost driver is the number of patients who received medical services during the reporting period. These costs will include salaries of junior medical staff, depreciation costs of medical equipment and devices used in the department, etc.

3. ALLOCATION OF COSTS IN AUXILIARY AND ADMINISTRATIVE SERVICE CENTERS

As for overhead costs outside the responsibility centers (costs of auxiliary medical units and administrative service units), we can use the ABC method to further transfer the costs formed in these units to the cost of the final medical service, which is formed in the responsibility center.

According to the ABC method, indirect costs Z_i are allocated to the service K_n on the basis of cost driver D . That is, all overhead costs formed in administrative units should be allocated by combining the cost factor (A) and cost driver (D). That is, we assume that for the provision of a certain medical service K , there are n indirect types of activities that accumulate aggregate costs Z ($i=1,..,n$) at the end of a period of time. That is, when planning for the next reporting year, the hospital management determines the total costs for each cost item, which must be included in the cost of the medical service in the future (Tables I-II).

Table I. Overhead Display Matrix by Responsibility Centers

Indirect cost item	Amount of overhead costs	
	<i>m</i>	
1	Z_{1m}	
2	Z_{2m}	
3	Z_{3m}	
...		
<i>n</i>	Z_{nm}	

Table II. Accounting data of the “Regional Clinical Psychiatric Hospital No. 3” KRC CNE regarding the amount of overhead costs

Indirect cost item	Amount of overhead costs, in 000, USD
Costs of non-medical raw materials and basic materials	314
Expenses for food for the patients	300
Fuel costs	11
Electricity costs	150
Sanitation costs	153
Heat supply costs	606
Administrative expenses	225

Table III. Matrix of Cost Factors

Cost factor	Responsibility centers				
	1	2	3	...	<i>m</i>
ΣA_1	A_{11}	A_{12}	A_{13}	...	A_{1m}
ΣA_2	A_{21}	A_{22}	A_{23}	...	A_{2m}
...				...	
ΣA_n	A_{n1}	A_{n2}	A_{n3}	...	A_{nm}

Table IV. The results of the agreement of experts’ opinions on the selection of the cost factor for the group of overhead costs

Indirect cost item	Cost factor item	Concordance coefficient	Pearson’s test, χ^2	Appropriateness
Costs of non-medical materials	Planned number of patients	0,644	15.45 \geq 7.81473	+
Expenses for food for the patients	Planned number of bed-days	0,7	16.8 \geq 7.81473	+
Fuel costs	Planned number of hospitalized patients	0,31	7.41 $<$ 7.81473	-
Electricity costs	Area of the premises	0,64	15.38 \geq 7.81473	+
Current repair costs	Area of the premises	0,64	15.38 \geq 7.81473	+
Heat supply costs	Area of the premises	0,64	15.38 \geq 7.81473	+
Administrative expenses	Planned number of bed-days	0.794	19.05 \geq 7.81473	+

There is also a cost factor, which is the basis combined with overhead costs, and the amount of overhead costs may depend on the value of the cost factor (Table III).

The complete collection of data used in ABC is almost impossible in some cases. In costing processes, collecting data for cost drivers is the most time-consuming and expensive step, especially when there are many activities and corresponding cost drivers. In the medical sector, this process is less labor-intensive than in manufacturing, since the most significant cost factor

for treatment and diagnostic services is the number of patients.

Since there is currently no clearly formalized evidence of the impact of a certain cost factor on the value of indirect costs in the scientific literature, we will use the judgment of 8 expert managers of medical institutions within the scope of this study. When asking for expert evaluations to calculate the dependencies between the cost factor and the value of the hospital’s indirect costs, we faced the problem of their consistency. We obtained a

Table V. Matrix of cost factors for «Regional Clinical Psychiatric Hospital No. 3» KRC CNE

Indirect cost item	Cost factor item	Cost factor sum	Cost factor values								
			1	2	3	4	5	6	7	8	9
Costs of non-medical materials	Planned number of patients	3024	30	700	200	400	350	420	320	252	352
Expenses for food for the patients	Planned number of bed-days	345	33	51	53	30	22	45	43	46	22
Fuel costs	Planned number of hospitalized patients	3024	30	700	200	400	350	420	320	252	352
Electricity costs	Area of the premises	2627	137	480	220	475	242	348	225	198	302
Current repair costs	Area of the premises	2627	137	480	220	475	242	348	225	198	302
Heat supply costs	Area of the premises	2627	137	480	220	475	242	348	225	198	302
Administrative expenses	Planned number of bed-days	345	33	51	53	30	22	45	43	46	22

Table VI. Calculation of cost drivers for each factor, the «Regional Clinical Psychiatric Hospital No. 3» KRC CNE

Indirect cost item	Indirect cost sum, USD	Cost factor item	Cost factor sum	Cost driver value
Costs of non-medical raw materials and basic materials	314108,1	Planned number of patients	3024	103,87
Expenses for food for the patients	300375,7	Planned number of bed-days	345	870,65
Fuel costs	11243,24	Planned number of hospitalized patients	3024	3,72
Electricity costs	149991,9	Area of the premises	2627	57,10
Sanitation costs	153097,3	Area of the premises	2627	58,28
Heat supply costs	606132,4	Area of the premises	2627	230,73
Administrative expenses	225400	Planned number of bed-days	345	653,33

comprehensive assessment of the consistency of experts' opinions using the concordance coefficient (general concordance coefficient). The results (Table IV) summarize the agreement of experts' opinions regarding the use of the cost factor for the distribution of overhead costs of a mental health care and psychosocial support institution. Matrix of cost factors for «Regional Clinical Psychiatric Hospital No. 3» KRC CNE is presented in Table V.

The cost drivers for each cost factor will be calculated according to the formula

$$d_n = Z_n / A_n$$

With a cost driver for each group of overhead costs, we can make the allocation of overhead costs by responsibility centers and calculate the cost per group of medical services provided in each responsibility center (Table VI).

With determined value of the cost factor for each cost center and the value of the cost driver for the group of overheads, it is possible to allocate costs by responsibility centers using the method of direct distribution (Tables VII-VIII).

That is, the largest share of indirect non-medical costs is accumulated by the Emergency Psychiatry Center: 18%.

Calculations showed that in the «Regional Clinical Psychiatric Hospital No. 3» KRC CNE, different responsibility centers accumulate different amounts of overhead costs, if we consider them through the prism of the cost factor, which was determined by selected experts in the medical field. Thus, 18% of the total annual overhead costs of the mental health care facility «Regional Clinical Psychiatric Hospital No. 3» KRC CNE should be allocated to medical services provided by the Emergency Psychiatry Center.

On analyzing the total number of patients treated by this facility in 2022, we can conclude that about 40% of the overhead costs were incurred for the treatment of patients with mental disorders caused by the use of psychoactive substances, and about 30% - for the treatment of patients with schizophrenia, schizotypal and delusional disorders (Table IX).

Thus, it is illogical to set a single tariff (in 2022, it was approximately \$330 USD) [6] for one treated case for all diagnoses treated in a mental health institution.

DISCUSSION

The existing system of reimbursement of costs for the provision of medical services determines a single tariff for the

Table VII. Determining the cost of service within the responsibility center

Indirect cost item	Indirect cost sum	Responsibility centers				
		1	2	3	...	m
1	Z_1	$\Sigma A_1 \times d_1$	$\Sigma A_2 \times d_1$	$\Sigma A_3 \times d_1$...	$\Sigma A_m \times d_1$
2	Z_2	$\Sigma A_1 \times d_2$	$\Sigma A_2 \times d_2$	$\Sigma A_3 \times d_2$...	$\Sigma A_m \times d_2$
3	Z_3	$\Sigma A_1 \times d_3$	$\Sigma A_2 \times d_3$	$\Sigma A_3 \times d_3$...	$\Sigma A_m \times d_3$
...
n	Z_n	$\Sigma A_1 \times d_n$	$\Sigma A_2 \times d_n$	$\Sigma A_3 \times d_n$...	$\Sigma A_m \times d_n$
Cost of service		S_1	S_2	S_3		S_m

Table VIII. The cost of services within the responsibility centers of the "Regional Clinical Psychiatric Hospital No. 3" KRC CNE

Indirect cost item	The cost of a service within the responsibility centers, 000 USD								
	1	2	3	4	5	6	7	8	9
Costs of non-medical materials	3,1	72,7	20,8	41,5	36,4	43,6	33,2	26,2	36,6
Expenses for food for the patients	28,7	44,4	46,1	26,1	19,2	39,2	37,4	40,0	19,2
Fuel costs	0,1	2,6	0,7	1,5	1,3	1,6	1,2	0,9	1,3
Electricity costs	7,8	27,4	12,6	27,1	13,8	19,9	12,8	11,3	17,2
Current repair costs	8,0	28,0	12,8	27,7	14,1	20,3	13,1	11,5	17,6
Heat supply costs	31,6	110,8	50,8	109,6	55,8	80,3	51,9	45,7	69,7
Administrative expenses	21,6	33,3	34,6	19,6	14,4	29,4	28,1	30,1	14,4
Cost	100,9	319,2	178,4	253,2	154,9	234,2	177,8	165,7	175,9
Share,%	6,0	18,0	10,0	14,0	9,0	13,0	10,0	9,0	10,0

Table IX. Distribution of costs of the Emergency Psychiatry Center by disorders of treated patients

Names of disorders	% to the total number of patients in the department	Total cost per medical service in 000 USD
Organic, including symptomatic	16,62	53,04
Caused by psychoactive substances	37,63	120,12
Schizophrenia, schizotypal and delusional disorders	28,99	92,55
Mood disorders (affective disorders)	4,70	14,99
Neurotic, stress-related and somatoform disorders	5,27	16,82
Syndromes of behavioral disorders associated with physiological disorders and physical factors	0,17	0,54
Mature personality and behavior disorders in adults	1,04	3,33
Mental retardation	5,58	17,80

provision of inpatient medical care in mental health care institutions. This hinders the creation of a reliable model of cost management in hospitals, since, as the results of our study show, the costs of treating some disorders are overstated, and on the contrary, understated for others.

The analysis of theoretical perspectives showed that scientists consider the ABC costing system an alternative to the traditional costing system [8-11] and a tool for cost analysis and control in the patient care chain [13-14]. One of the disadvantages of this system is that it is time-consuming [15]. However, in health care institutions that consume a significant amount of budgetary resources, its benefits override the costs of its implementation [12-13].

The results of the calculations showed that the calculation of the cost of a separate medical service, that is, the treatment of a certain disorder, can be carried out in several stages. At the first stage, one should determine the centers of formation of costs of the main activity. These are the units of the hospital within which medical services are provided. At the second stage, direct costs can be calculated within the cost center for each patient based on the hospital's existing data base. We suggest that only the salary of the attending physician and the costs of medicines provided under the Medical Guarantee Program, are included in the direct costs. All other costs are overhead costs and need to be apportioned. Those overheads that arise within the cost

center should be allocated to the number of treated patients, and overheads that arise in administrative and service units should be allocated using the ABC system.

CONCLUSIONS

The ABC provides inpatient psychiatric care with a tool to estimate the cost of treatment for each category of patients. This information is especially important for setting tariffs for psychiatric care and monitoring the use of resources for patient treatment. However, in order to get a complete overview of costs for different categories of patients, it is necessary to understand where costs are generated and what factors influence the amount of overhead non-medical costs throughout

the treatment process. The most important and most expensive element of the implementation of the ABC system is the determination of cost factors. In this study, we used the opinions of experts and for each item of overheads, we chose the appropriate factor that made it possible to calculate the cost driver and distribute the overheads by cost centers. On obtaining the values of the cost drivers for each cost center in the financial plan, we can calculate the cost of treating patients for each mental health disorder or for a group of related diagnoses. This creates a basis for improving the efficiency of resource use throughout the entire chain of inpatient psychiatric care and shows that ABC could be used in mental health and psychosocial support institutions in Ukraine.

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ORIGINAL ARTICLE

CONTEXTUAL FACTORS ASSOCIATED WITH CERVICAL CANCER

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ABSTRACT

The aim: The aim of the study is to determine the risk factors associated with cervical cancer.**Materials and methods:** 195 participants took part in the study, including 100 cases and 95 healthy people; participation was voluntary. The association of risk factors with cervical cancer was tested using the chi-square test and odds ratio. The use of these methods has been agreed with the ethics commission of the Poltava State Medical University and meets the requirements of the Declaration of Helsinki.**Results:** Women who live in the village were 64.6% less likely to get sick than those who live in the city, those who assessed their financial situation as unsatisfactory 2.1 times more often have a chance of CC. Childless women are 2.4 times more likely to get sick than those who have children.**Conclusions:** Various groups of contextual risk factors for cervical cancer have been established. The main ones are behavioral factors related to the diet and diet; socio-economic factors and medical factors. It was also established that the place of residence of women influences the probability of the occurrence of RSM.**KEY WORDS:** oncogynecology, visual forms of cancer, risk factors, organization of medical care

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INTRODUCTION

Cervical cancer is the leading type of cancer among women both in Ukraine and in the global community. During the last decades, in the general structure of oncological diseases, cervical cancer remains among the five most common oncological diseases, ranks second position after breast cancer among malignant neoplasms of the female reproductive system and ranks fourth in the prevalence of cancer among women aged 18-29 [1].

Cervical cancer ranks seventh among the most common types of cancer among women in the WHO European Region, and its share in the structure of the total cancer incidence is about 3.8%. About 300,000 women die from cervical cancer every year. It is relatively rare in high-income countries where screening programs are widely implemented and operating [2].

About 500,000 new cases of cervical cancer are registered and 300,000 women die from this disease annually. The increase in the frequency of CC among women of childbearing age is an extremely acute problem, since this contingent of patients represents not only a reproductively significant part of the population, but also a socially active group [3].

According to the Center for Medical Statistics of the Ministry of Health of Ukraine, in 2020, 12,948 cases of cancer

of the female genital organs were registered in Ukraine, of which 3,319 were cervical cancer.

In 2020, WHO presented the Global Strategy to Accelerate Cervical Cancer Elimination. Through the introduction of vaccination, screening and timely treatment, it is expected to reduce the incidence by 40% by the middle of the century.

In Ukraine, 1 out of 3 cases of cervical cancer is diagnosed in the neglected stages of the disease, which means that it is no longer possible to save patients even with the use of the most advanced treatment methods.

Therefore, conducting new studies aimed at obtaining results about the medical and social risk factors for the development of CC and their in-depth scientific analysis is a solution to the current problem related to women's reproductive health [4].

THE AIM

The aim of the study is to determine the risk factors associated with cervical cancer.

MATERIALS AND METHODS

The research was conducted using a quantitative method. A case-control study design was used to evaluate

Table I. Socio-demographic profile of women

Changeable	The number of women with CC n=100 abs (%)	Number of healthy women n=95 abs (%)	$\chi^2(df)$, p	Mean \pm standard deviation
Age				
Up to 30 years	2 (2,0)	18 (18,9)	35,8 <0,001	
31-40	10 (10,0)	24 (25,3)		
41-50	24 (24,0)	27 (28,4)		
51-60	40 (40,0)	18 (18,9)		
61-70	20 (20,0)	6 (6,3)		
71 and older	4 (4,0)	2 (2,1)		
Education				
Average	13 (13,0)	48 (50,5)	39,0 <0,001	
Higher	62 (62,0)	22 (23,2)		
Medium special	25 (25,0)	25 (26,3)		
Place of residence				
Rural resident	46 (46,0)	84 (88,4)	39,4 <0,001	
City resident	54 (54,0)	11 (11,6)		
Employment				
Does not work	59 (59,0)	53 (55,8)	0,2 0,6	48,4 \pm 12,5
Working	41 (41,0)	42 (44,2)		
Working conditions				
Light	15 (15,0)	28 (29,5)	9,7 0,008	
Average	73 (73,0)	49 (51,6)		
Heavy	12 (12,0)	18 (18,9)		
Financial position				
Less than 5000	59 (59,0)	41 (43,2)	19,1 <0,001	
5000-10 000	19 (19,0)	8 (8,4)		
11 000- 15 000	3 (3,0)	1 (1,1)		
More than 15,000	19 (19,0)	45 (47,3)		
Assessment of financial position				
Unsatisfactory	68 (68,0)	48 (50,5)	6,1 0,014	
Satisfactory	32 (32,0)	47 (49,5)		

risk factors. Women who came for an oncological examination to a gynecologist at the Poltava Regional Clinical Hospital were asked to fill out a questionnaire that took into account the risk factors for cervical cancer. The target population consisted of women aged from 20 (min=20) to 85 (max = 85) years. 195 participants took part in the study, including 100 cases and 95 healthy people; participation was voluntary. The inclusion criteria for the cases were women with a diagnosis of cervical cancer. The criteria for inclusion in the control group were women who had not been diagnosed with any cancer and who corresponded to the group of cases by age.

Data were collected using a questionnaire, which was divided into blocks: demographic, economic, behavior-

al, history of reproductive health, heredity and relation to preventive examinations.

Data entry and statistical analysis were performed using R Statistics.

Data were analyzed using descriptive statistics, i.e. mean, median, mode and standard deviation. Baseline categorical and continuous variables were compared between groups using the chi-square test with Yate's correction. The association of risk factors with cervical cancer was tested using the chi-square test and odds ratio. As for eating behavior, consumption of products 1-2 times a week was considered infrequent, and 3 or more - regular consumption. Risk factors that significantly increase the likelihood of cervical cancer have been analyzed. $p < 0.05$ was considered statistically significant.

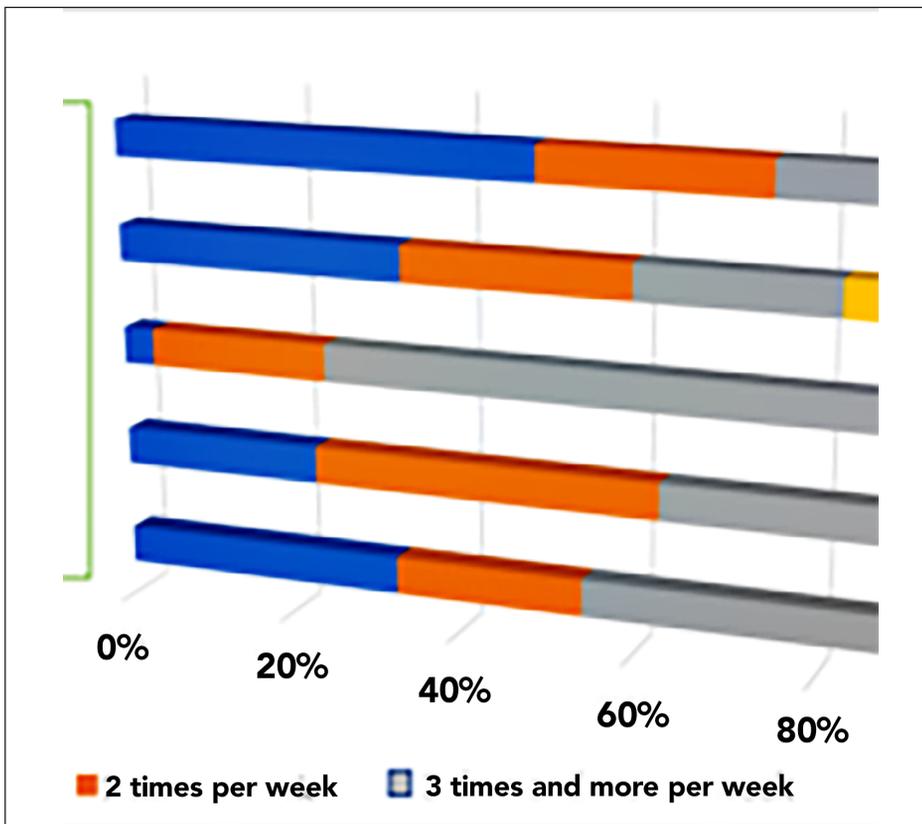


Fig.1. Frequency of food consumption.

RESULTS

Cervical cancer is a cancer caused by an infectious disease [6]. In particular, two types of HPV, HPV 16 and 18, account for approximately 70% of cervical cancer cases [7]. Despite the fact that HPV-infection is an obligatory cause in the etiology of cervical cancer, HPV-infection alone is not a sufficient cause of the occurrence of cases [8]. Several epidemiological studies have shown the role of various demographic, sexual and reproductive factors in the development of HPV infection in cervical carcinoma [9 - 14].

Despite declining trends in the incidence and mortality of cervical cancer, it remains a serious health problem [15,16]. Therefore, conducting new studies aimed at obtaining results on the medical and social risk factors for the development of cervical cancer (CC) and their in-depth scientific analysis is a solution to the current problem related to the reproductive health of women.

Table I shows that the average age of the respondents of the two groups is 48.4 ± 12.5 years. A significant majority of the participants had a higher education, average working conditions, a minimum income of UAH 5,000, were residents of rural areas and assessed their financial situation as unsatisfactory.

Table II shows the results of the odds ratio assessment. We analyzed prognostic factors, such as socio-demographic, physical development, behavioral, use of medical services, concomitant diseases, heredity.

Socio-demographic factors. Women who have assessed their financial situation as unsatisfactory and those who are childless are more likely to get CC. In women living in rural areas, there was significant association with CC ($p < 0.001$), but the odds ratio was less than one.

Physical development. In women with increased BMI, the risk of cervical cancer probably increased by 2 times.

Behavioral factors. The odds ratio was higher in women who did not adhere to the diet.

Use of medical services. Women who did not visit prophylactic examinations regularly and those who had a history of abortions had a higher chance of developing CC.

Associated diseases. Among concomitant diseases, inflammatory processes of the genital organs served as a risk for the development of an oncological process in the uterus.

Heredity was important only when one of the relatives had CC and increased the risk of developing the disease by as much as 80 times.

Cervical cancer has a high mortality rate among women and worsens their lives. Identifying the most important factors of cancer is the most important task of the prevention strategy and can even help in early diagnosis. Among the factors that cannot be overlooked is the influence of diet. Therefore, the next step of the study was to examine the behavioral factors related to

Table II. Associations between risk factors and cervical cancer

Questionnaire questions	The number of women with CC (n = 100) abs (%)	Number of healthy women (n = 95) abs (%)	Odds ratio (DI 95%)	χ^2	P
Socio-demographic factors					
Place of residence: village (n = 130)	46 (35,4)	84 (64,6)	0,112 (0,53-0,23)	39,4	<0,001
Place of residence: city (n = 65)	54 (83,1)	11 (16,9)			
Do you work: yes (n=112)	59 (52,7)	53 (47,3)	1,140 (0,65-2,01)	0,2	0,379
Do you work: no (n=83)	41 (49,4)	42 (50,6)			
Higher Education (n=144)	74 (51,4)	70 (48,6)	1,016 (0,54-1,93)	0,003	0,545
Secondary education (n=51)	26 (51,0)	25 (49,0)			
The financial position is unsatisfactory (n=116)	68 (56,8)	48 (41,4)	2,081 (1,16-3,72)	6,1	0,010
The financial position is satisfactory (n=79)	32 (40,5)	47 (59,5)			
There are no children (n=35)	24 (68,6)	11 (31,4)	2,411 (1,11-5,25)	5,1	0,018
There are children (n=160)	76 (47,5)	84 (52,5)			
Physical development					
Increased BMI	70 (58,8)	49 (41,2)	2,19 (1,20-3,90)	6,9	0,006
BMI is within normal limits (19-24)	30 (39,5)	46 (60,5)			
Behavioral factors					
Diet: no (n=152)	73 (48,0)	79 (52,0)	0,548 (0,27-1,10)	2,9	0,062
Diet: yes (n=43)	27 (62,8)	16 (37,2)			
Food regime: no (n=65)	43 (66,2)	22 (33,8)	2,503 (1,35-4,65)	8,6	0,003
Food regime: yes (n=130)	57 (43,8)	73 (56,2)			
Smokes (n=39)	22 (56,4)	17 (43,6)	1,294 (0,64-2,62)	0,5	0,296
Does not smoke (n=156)	78 (50)	78 (50)			
Use of medical services					
Pro view: no (n=98)	62 (63,3)	36 (36,7)	2,674 (1,50-4,77)	11,3	0,001
Pro view: yes (n=97)	38 (39,2)	59 (60,8)			
There was an abortion (n=123)	81 (65,9)	42 (34,1)	5,380 (2,83-10,23)	28,3	<0,001
There were no abortions (n=72)	19 (26,4)	53 (73,6)			
She used oral contraceptives (n=24)	14 (58,3)	10 (41,7)	1,384 (0,58-3,29)	0,5	0,302
She did not use oral contraceptives (n=171)	86 (50,3)	85 (49,7)			
Associated diseases					
Inflammatory processes of genital organs: yes (n=140)	99 (70,7)	41 (29,3)	130,390 (17,45-174,35)	75,0	<0,001
Inflammatory processes of genital organs: no (n=55)	1 (1,8)	54 (98,2)			
Endocrine diseases: no (n=172)	88 (51,2)	84 (48,8)	0,960 (0,40-2,30)	0,008	0,553
Endocrine diseases: yes (n=23)	12 (52,2)	11 (47,8)			
Heredity					
Did any of your relatives suffer from CC: yes (n=47)	46 (97,9)	1 (2,1)	80,074 (10,74-97,15)	53,8	<0,001
Did any of the relatives suffer from CC: no (n=148)	54 (36,5)	94 (63,5)			
Did any of your relatives suffer from breast cancer: yes (n=4)	0 (0,0)	4 (100)	2,099 (1,81-2,44)	4,2	0,055
Did any of the relatives suffer from breast cancer: no (n=191)	100 (52,4)	91 (47,6)			

Table III. Associations between eating habits and cervical cancer

Questionnaire questions	The number of women with CC (n = 100) abs (%)	Number of healthy women (n = 95) abs (%)	Odds ratio (DI 95%)	χ^2	P
Fish consumption: does not consume or once a week (n = 153)	86 (56,2)	67 (43,8)	2,567 (1,25-5,26)	6,902	0,007
Eating fish: two to three times or more (n = 42)	14 (33,3)	28 (48,7)			
Meat: three or more (n = 48)	69 (69,0)	22 (23,2)	7,3 (3,90-13,90)	41,135	<0,001
Meat: up to two times (n = 147)	31 (31,0)	73 (76,8)			
Vegetables: rarely (n = 48)	26 (54,2)	22 (45,8)	1,166 (0,61-2,24)	0,212	0,385
Vegetables: often (n = 147)	74 (50,3)	73 (49,7)			
Fruits: rarely (n = 106)	86 (81,1)	20 (18,9)	23,036 (10,88-48,76)	82,830	<0,001
Fruits: often (n = 89)	14 (15,7)	75 (84,3)			
Dairy products: rarely (n = 125)	73 (58,4)	52 (41,6)	2,236 (1,23-4,07)	7,062	0,006

nutrition that affect cervical cancer. Figure 1 shows that most women eat vegetables three times a week and fish - almost half a week or less.

As shown in Table III, those who do not often consume fish, fruits, and dairy products, but instead often consume meat products, have a greater chance of developing CC.

DISCUSSION

The conducted study showed that an unsatisfactory financial situation, childlessness, increased BMI, non-compliance with the diet, abortions and inflammatory processes of the genital organs in the anamnesis, irregular prophylactic examinations, heredity for CC increased the chances of its occurrence.

Unsatisfactory financial status was shown as a risk factor for PCM in the study by Shield TS et al., and in addition, such risk factors as low level of education and genital infection were found in this study. In our questionnaire, it was not specified exactly which inflammatory processes of the genital organs were present in women, and it can be concluded that inflammation of both infectious and non-infectious genesis are risk factors for CC [14,17].

Irregular preventive examinations indicate women’s ignorance of the need to undergo them, which was consistent with the study conducted by Tebeu PM at al. on the relationship to CC and awareness of it. The fact that women from rural areas had a lower chance of developing RSM may also indicate low early detection, untimely diagnosis due, on the one hand, to lack of awareness of the need

for preventive examinations, and on the other hand, due to lower accessibility to medical care in the village. In addition, if a woman has probable signs of CC, she may not go to a health care facility for many reasons, such as inactivity, poor socioeconomic status, lack of transportation, financial problems, etc. [18]. An increased BMI was one of the risk factors for CC. In a meta-analysis by Poorolajal J et al, it was shown that overweight is not associated with an increased risk of cervical cancer, but obesity is weakly associated with an increased risk of cervical cancer [19].

Analysis of food behavior outlined that the consumption of meat more than 3 times a week, fruit and dairy products - twice or less a week, and neglecting the consumption of fish are risk factors for CC. A study conducted by Barchitta et al. shows that a high intake of red and processed meat, sauces, chips and snacks with a low intake of olive oil in a Western diet was associated with a higher risk of HPV. In contrast, a Mediterranean diet consisting of vegetables, legumes, fruits and nuts, cereals, fish and a high ratio of unsaturated lipids to saturated lipids had a lower risk of CC [20, 21].

Thus, the identified risk factors will later become factors for justifying the need to develop a model for early detection and prevention of cervical cancer.

CONCLUSIONS

As a result of the research, various groups of factors that increase the risk of cervical cancer in women were established, namely:

1. Factors related to the diet. Women who consumed an insufficient amount of fruit, neglected the consumption of fish and dairy products, and preferred meat in their diet - had a greater chance of developing PCM.
 2. Socio-economic factors, such as a low level of education, lack of awareness about early detection of cervical cancer, unsatisfactory financial situation of women increased the risk of cervical cancer.
 3. At the same time, it was established that medico-demographic factors, such as the absence of children, a history of abortion, hereditary predisposition (that is, the presence of this oncological disease in a woman's relatives) increased the chances of PCM.
- It should be noted separately that the place of residence affects the probability of cervical cancer. According to the results of the survey, women living in rural areas had a lower chance of developing PCM, and this is most likely due to low awareness and seeking medical help by women.

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REVIEW ARTICLE

TOTAL HIP JOINT REPLACEMENT USING A CUSTOM TRIFLANGE ACETABULAR COMPONENT (LITERATURE REVIEW)

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ABSTRACT

The aim: The purpose of the article is to analyze the ways of solving the problem of revision hip joint replacement. The article discusses the methods of treatment using a custom triflange acetabular component.

Materials and methods: The analysis of 37 literary sources includes a discussion of the features of the use of individual triflange acetabular components and errors in revision hip arthroplasty, which are associated with various factors.

Conclusions: A review of studies devoted to the use of custom triflange acetabular components confirms the effectiveness in the early postoperative period in the treatment of critical acetabular defects and pelvic ring discontinuity. The CTAC use is particularly relevant in case of the pelvic ring disintegration, as it provides for the appropriate endoprosthesis adaptation with the healthy bone, as well as for the bone defects plastics and recovery of the hip joint biomechanics. So, the use of individual constructions is indicated for the patients with significant bone mass loss, where augment adaptation and adjustment is impossible. This method is used more often when there is no other alternative. Research results showed a trend that special three-flange components of the acetabulum have better long-term results compared to traditional standard components for large bone defects. Improving production and increasing the number of CTACs should reduce their cost. In summary, the custom triflange acetabulum components provide a personalized secure fit that can reduce the risk of complications and improve patient outcomes. In summary, the triple-flange acetabulum components provide a personalized secure fit that can reduce the risk of complications and improve patient outcomes.

KEY WORDS: total hip replacement, custom triflange acetabular component, hip arthroplasty, hip replacement complications

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INTRODUCTION

Finding the effective surgical treatment method of the hip joint injuries and diseases represents a key challenge of modern traumatology and orthopedics. Numerous authors support the idea that the best and often the only possible treatment method for such patients is the pelvis joint endoprosthesis [1]. According to Learmonth I. et al., this operation is "the most successful operation of the 20th century". This surgical intervention provides for the restored joint function, improved life quality and, regarding the working-age patients group, is helpful for resuming the working activity of the last [1-5].

The operation is done to patients of almost any age group, ranging from the teenagers to elderly people [2].

According to the WHO, by 2050, diseases of bones and joint will be observed in 25% of the world population [1]. Nowadays, over 1.5mln hip joint endoprosthesis operations are performed annually [6]. In the USA, scientists predict about 572000 operations a year [7]. An increase in the hip joint endoprosthesis percentage directly leads to increase in the revision interventions worldwide. Thus, in the USA the share of the hip joint endoprosthesis operations is expected to increase by 137%, in the period of 2005-2050 [7]. The specific gravity of such operations within the general structure of the hip joint endoprosthesis makes up from 13 till 18% [8].

Unfortunately, not all patients are eligible for the revision endoprosthesis after the wear or loosening of the endoprosthesis. This is related to numerous factors:

Revision endoprosthetics is a complicated operative intervention which should be performed by an experienced orthopedist, and the need for such operations significantly exceeds possibility of being operated. High cost of the revision interventions, often postponing operations due to low affordability of it, late patients' referrals, when in early referral the problem could be eliminated before clinical symptoms appear – all these lead to increase in severe acetabulum destruction incidence, with vast bone tissue loss and considerable acetabular defects. This makes a significant problem for the hip joint revision operations, making such operations most difficult in endoprosthetic surgery [5, 9]. Some authors state that the incidence of huge acetabular defects is 8.5 % among the revisions of the hip joint [10] and 27 % among the acetabulum revisions [11].

According to the Danish National Endoprosthetic Center, the 3rd class defects by Paprosky and the pelvic ring disintegration are increasing by both absolute and relative values, as well as the expenses for the total endoprosthetic revision of the hip joint. As for the Norwegian Register, the data of acetabular defects have remained steady for the Paprosky 3A and Paprosky 3B defects for the last 5 years [12].

Several suggestions for the reconstruction of the acetabulum stages 2 and 3 by Paprosky, have been described, which include structural allografts [13,14,15], augments and module components from trabecular metal [16], anti-protrusion cages, and the various cup-cage systems [17-22].

Quite often, the standard porous component constructions are not sufficient for the revision endoprosthetics, which results in indications for the individual acetabular components use (CTAC-custom triflange acetabular component). C.C. Berasi et al. state that these indications include: 1) history of faulty use of augments or bands; 2) huge defects of the acetabular part with the disintegration of the pelvic ring; 3) multiple operations on the hip joint, resulting in bone defects and bone deficiency, which are not to be reconstructed in other ways [23].

The CTAC use is particularly relevant in case of the pelvic ring disintegration, as it provides for the appropriate endoprosthesis adaptation with the healthy bone, as well as for the bone defects plastics and recovery of the hip joint biomechanics [12, 24-26].

The use of the CTAC-acetabular systems is a modern technology, which has been used for the last 5 years, both in Ukraine and neighboring countries. But this method is already introduced into the surgical practice of numerous countries, being used for 15-20 years. During this time, practical experience regarding surgical facilities, method effectiveness, postoperative

complications, and economic benefits of the CTAC has been gained.

The literature review, presented in this article, is dedicated to the data analysis.

THE AIM

The purpose of the article is to analyze the ways of solving the problem of revision hip joint replacement. The article discusses the methods of treatment using a custom triflange acetabular component.

MATERIALS AND METHODS

The analysis of 37 literary sources includes a discussion of the features of the use of individual triflange acetabular components and errors in revision hip arthroplasty, which are associated with various factors.

REVIEW AND DISCUSSION

PECULIARITIES OF SURGICAL TECHNIQUE

The technique of the hip joint revision endoprosthetics with the CATC is usually standard, characterized by: more broadly exposed bones which make the acetabulum, for comfort view of a defect and surfaces used for adjustment. After the standard removal of the non-stable primary graft and clearing the bone bed from the scars, CTAC is positioned. The place for the individual acetabular components pubic and ischial flange is prepared by cautious subperiosteal shedding of soft tissues. The vascular and nervous structures must not be damaged. The expressed bone defects need for the bone plastics. After the CATC is positioned, it is attached, the procedure starts with the ischial flange and needs 9-15 screws. Eccentric, lateralized and combined CATC versions may be used to reach the required soft tissue tension and provide for the endoprosthesis components' stable position.

Hourscht C. announced their results after the average period of 4.5 years, after having performed 26 triflange reconstructions, which included three disintegrated pelvic ring cases (AAOS Type-IV) [27]. Two of these three hip joints with the disintegrated pelvic ring were characterized by loose ischial screws, with lost ischial flange adjustment. The screws were not broken. In the Berasi C.C. type, one patient with such faulty ischial flange adjustment wore a stable graft for 11 years [23].

So, during the arrangement of the CTAC, special attention is paid to the ischial flange attachment. To prevent the screws from loosening, which may result from the bone tissue quality deterioration, the screws

are primarily twisted in the ischial bone, which provides to pull the CTAC downwards and provide contact of the flange with a bone tissue. An alternative to the screw attachment improvement is filling the ischium bone defect with cement before the screws are introduced. Some authors consider the option of improving attachment by using blocking screws [6,28, 29].

EFFECTIVENESS

Application of the CTAC has shown its high effectiveness in early postoperative period. In studies of numerous researchers, by the Harris scale, the difference in values before the operation and in a year after it ranges within: 36-46 till 75-80 points [30-32].

The frequency of revisions after the CTAC use related to massive defects of the acetabulum (3B type Paprovsky), according to various authors, ranges from 7 to 13.5 %. An increase in revisions, shown in the study of Barlow B.T. et al. was related to the mistakes during locating endoprosthesis (shifting the rotation center for over 2 cm) [20, 21, 27, 29, 33-36].

Different statistical data have been revealed using the CTAC for the pelvic ring dislocation. Thus, in the studies by De Boer D.K. et al. and Taunton M.J. et al., the frequency of revisions was 30 and 35 % [22,28], in the period (123 months and 76 months respectively) after the operation.

A potential advantage of the CTAC is possibility of precise positioning and attachment of the endoprosthesis acetabular component. The results of this method vary with different researchers.

C.C. Berasi et al. have stated only 4 (14.3 %) repeated revisions out of 28 cases of the CTAC use, in patients with the acetabular defects type 3 B by Paprovsky, observed during the period of 4.5 years. The repeated revisions were caused by: two cases of periprosthes infection and one case of the acetabular component loosening. Here the authors suggest, that the CTAC used with the augments or anti-protrusion cages, for the severe acetabular defects, with disintegrated pelvic ring, will be more effective [23].

The authors of another analysis defined that compared to the alternative treatment options (anti-protrusion constructions with or without bone plastic, trabecular metal constructions), revisions incidence increases twice with the CTAC [34].

The scientists think that the causes of relatively high frequency of revisions, associated with the CTAC, may be explained as follows:

1. Surgeons use the CTAC in cases of impossible reconstruction with a simple graft, which usually is not complicated cases.

2. The CTAC effectiveness estimation in a long-term perspective includes the first individual grafts generation, in which biointegration ability of lower than in the modern constructions, made from the trabecular metal.
3. Using the available data, it is hard to compare the treatment outcomes, which are connected with non-homogenous bone defects in various studies and their imperfect classification. The authors suggest estimating the treatment results of the pelvic ring disintegration and the 3B defects in separate groups.
4. Considering that all the researchers have observed a dozen or more patients, we could assume that the CTAC is the first experience of surgeons, and so, the complications percentage is connected with the introduction of new grafts and different techniques of their adjustment [10, 35].

According to the newly published metaanalysis, the average frequency of revisions observed with treatment of the huge acetabular defects, with the CTAC was 3.8-30.3%. The analysis includes data from 193 patients and 5 studies of the significance level IV [6,8, 28, 32], with the average of revisions, equaling 7.8 %, and the complications percentage - 22 % during 5 years [27, 31]. Nonetheless these values increased to 30 and 35% respectively in studies older than 10 years [28, 29].

POSTOPERATIVE COMPLICATIONS

The most frequent complication which did not require revision, according to the publications, is represented with dislocations. The percentage of dislocations differs between different authors, from 0 to 6.4% [27, 28, 29] to 33 % [36]. M. Citak et al. related a large proportion of postoperative dislocations to the hip joint repeated operative interventions and bone tissue deficiency in the ischiac muscle attachment region, accompanied by muscle misbalance. [36]. The greater trochanter dislocation after the periprosthes fracture, following the osteolysis or injury, according to MJ. Taunton et al., may be a risk factor for the recurrent endoprosthesis head dislocations when using CTAC. A precise inclination and anteversion as well as the cup design minimize the dislocation risk after attaching the CTAC [22].

As BT. Barlow et al. suggest that extreme verticalization of the endoprosthesis acetabular components is a typical mistake in restoring the acetabulum with segmental defects, which soon leads to the recurrent dislocations [21]. The choice method for recurrent dislocations is using the head with double mobility [8], and use of connected inlays without the acetabular component biointegration increases risk of loosening the acetabular construction.

The next complication is represented with the nerve damage, which represents the minor complications with the incidence of 4 - 8 % [27, 29, 31, 37].

ECONOMIC JUSTIFICATION OF THE CTAC USE

Upon the review of available literature sources, the authors have found a small portion of publications dedicated to the economic effectiveness of the CTAC use. The CTAC advantages include small operation time and, appropriately, decrease in the postoperative complications incidence. The final cost of computer tomography, modeling and producing the CTAC, according to De Boer D.K., may exceed the cost of operation itself. The price of a triflange cup in 2006 was about US \$ 8500. Improved clinical results justify such a high price [28].

The CTAC price ranges from that compared to analogical alternatives to the exceeding alternative methods by 36-46%. Taunton M.J in his studies estimated the CTAC cost, including the cup, screws, polyethylene inlay and the production process as equal to \$12500. At the same time, the construction made from the tantalum cup, screws, anti-protrusion cage and polyethylene inlay costs \$11250. If extra two augments from porous metal were used, the construction cost \$14500 [29]. Wyatt M.C. states that the cost of individual implants MOBE-

LIFE was £13,000, OSSIS – £11,000 and TMT Cup-Cage – £7,000 [12]. One should note that the study data do not consider the effect of method on the patients' life quality. It should be emphasized that the authors have not found any studies of the "cost-effectiveness" analysis. So, it is early to make any conclusions about the economic effectiveness of the method.

CONCLUSIONS

A review of the publications dedicated to the CTAC use proved its high effectiveness in an early postoperative period, used for the treatment of the acetabular critical defects and pelvic ring discontinuity, where this method is a choice method and alternative methods are impossible to use. Other methods require maximum adaptation of constructions and allografts to the defect borders and the acetabular bed. With the CTAC use, the osseous bed adaptation is minimum. So, the use of individual constructions is indicated for the patients with significant bone mass loss, where augment adaptation is impossible.

As for the cost and economic effectiveness, the authors suppose that optimizing the production and increasing the production of constructions should affect their cost (toward lower price). The authors recommend this method for broader use in Ukraine.

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REVIEW ARTICLE

CONCEPTUAL DIRECTIONS OF THE ORGANIZATION OF DENTAL CARE FOR THE POPULATION OF UKRAINE AND EUROPEAN COUNTRIES

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ABSTRACT

The aim: Conducting a scientific analysis of domestic and foreign sources of information regarding modern conceptual views on the organization of dental care for the population of Ukraine and European countries.

Materials and methods: Used methods of historical-bibliographic and systematic approach. The search for literary sources was carried out in four main scientific databases: Scopus, PubMed, BVS, and Scielo. The review included original articles, research, and official recommendations from medical associations.

Conclusions: Scientific analysis confirmed the expediency of reforming the dental service and substantiated the need to improve dental care as well as the dental care management system to increase medical, social, and economic efficiency.

KEY WORDS: health care, reform, dental facilities per capita, dentists per capita, availability of dental care

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INTRODUCTION

One of the components of the health care system in Ukraine is dental care, the main mission of which is to preserve and restore the dental health of the population and improve the quality of life. Modern conditions for the organization of dental care require fundamentally new approaches to ensuring the quality of services. Given this, the process of reforming the medical industry affected the dental field as well. Prevention implemented within national strategies is a real mechanism for solving global problems related to dental diseases and their risk factors. European and world experience shows that, first of all, it is necessary to rebuild sanitary and educational activities on the model of economically developed countries. Using the example of some European countries, let us try to highlight some conceptual directions of the organization of dental care for the population.

THE AIM

Conducting a scientific analysis of domestic and foreign sources of information regarding modern conceptual views on the organization of dental care for the population of Ukraine and European countries.

MATERIALS AND METHODS

Used methods of historical-bibliographic and systematic approach. The search for literary sources was carried out in four main scientific databases: Scopus, PubMed, BVS, and Scielo. The review included original articles, research, and official recommendations from medical associations.

REVIEW AND DISCUSSION

During three decades, the political vector of the state's development, economy, and Ukrainian society changed. The main basis for the development of the health care system of the population of Ukraine is the implementation of the main provisions of the Constitution and laws of Ukraine regarding the provision of affordable qualified medical care to every citizen of Ukraine. The last ten years were characterized by radical processes of healthcare transformation, the introduction of new mechanisms of funding and management of the industry, capable of providing medical care for all citizens of Ukraine at the level of developed European states and patient-oriented [1-4]

Significant changes also took place in the dental field. The dynamic development of dentistry, innovative

technologies, and their implementation in practical health care [5], on the one hand, the increase in patient requirements for the aesthetic component of dental services focused on high-value technologies, on the other hand, all this led to a situation where the state was unable to fully finance the dental industry.

Analyzing the scientific sources of European countries, it can be stated that the global practice of providing dental care to the population is developing at an active pace in the direction of attracting patients to pay for dental services. In most European countries, national systems of dental care consist of three components: private, public, and insurance. In the economically developed countries of the world, there are two models of health insurance: Bismarck and Beveridge. Compulsory insurance programs of 28 countries of the world include the provision of dental services in various volumes, mainly preventive and treatment of uncomplicated caries [6-8]

In the countries of Western Europe, quality assurance standards developed following ISO 9001 and 9002 [9] began to be widely implemented in health care. In the developed countries of the world, dental service consumes approximately 6-8% or more of all financial resources of the health care system (in Ukraine, less than 2%). Dental care in Western countries is largely based on preventive work. Dental care is associated with large financial costs.

The system of medical care in European countries should be considered considering the social characteristics, the economic policy of the state, and entrepreneurship in the largest countries of Western Europe - Germany, France, Italy, as well as Sweden and Switzerland. The specificity of the European model as a whole lies in the fact that post-war European capitalism managed to transform its political, economic, and social institutions in the direction of creating a socially oriented, economically efficient version of a mixed economy. The most important principle of the European model is «democratic evolutionism» - a gradual, step-by-step solution to problems arising in the process of global changes, based on negotiations and consensus.

At the end of the last century, there were reforms in dentistry in the Netherlands, Great Britain, Germany, and Sweden, which were aimed at introducing elements of market relations into the interaction between subjects of public health care [10].

This ideology also played an important role in reforming dentistry in Spain, Italy, Israel, Finland, Norway, and New Zealand [6, 9, 11, 12]. The essence of the proposed new principles for the organization of public financing of the health care system was reduced to

three key provisions: separation of functions of buyers and producers of medical services; competition of producers; buyer competition. In the late 1980s, standards were developed that are now used in the certification of quality assurance systems. Quality assurance, under ISO 9001 and 9002 standards, should ensure appropriate trust between the manufacturer of goods and services and the client [9, 13]. Since the mid-1990s, this system has been widely implemented in medicine and health care in Western European countries. The development of national health care and medical insurance programs in various European countries is dialectically interconnected with the political, social, and economic conditions that have developed in these countries [9, 14, 15]. Programs differ in the main sources of funding and forms of ownership (state, public, private), insurance conditions, the list and volume of medical services provided within the limits of this program, organizational forms of their provision, and other parameters. Management of funds is controlled by state structures. The difference between the formation of budgetary and social insurance funds lies in their target orientation. Insurance funds are aimed at a certain circle of people who participate in the insurance program. As a result, contributions come from three main sources: the state, entrepreneurs, and workers (these contributions go directly to healthcare needs and are used purposefully) [9]. The targeted nature of funding allows for a more flexible response to changing public needs by making appropriate adjustments to the targeted health insurance taxation system. Another advantage of the health insurance system is the decentralization of financial management, which contributes to the strengthening of the local healthcare resource base [11].

In European countries, approximately 6-8% of the national budget of the health care system is currently spent on dental care, and a total of about 420,000 dentists and at least as many auxiliary medical personnel work in dental services. The main features of European dentistry include the availability of dental services and the quality of services, the integration of dental and general health care services, the rational basis of dental care, the use of the team method of work in dental services, high-tech dental office equipment and its use, sociological research and prevention among the population. However, the differences in the availability of medical care, the levels of coverage of the population by medical insurance, the conditions for the provision of medical services, and the types of insurance programs are quite significant. Thus, compulsory insurance programs in 28 countries of the world (Germany, England, Italy, Belgium, the United States of America, Holland,

Finland, Denmark, etc.) include in various volumes the provision of dental services, mainly preventive and treatment of uncomplicated caries [9, 16-18].

Dentistry in Germany is among the best in the world and is one of the most developed areas of medical care in the country. Dentistry in Germany is characterized by an individual approach to the patient and the use of the latest achievements of technical and scientific progress [19], which attracts patients from many countries. Comprehensive consultations, accurate diagnosis, effective prevention and treatment of dental diseases, aesthetic dentistry, dental implants, and prosthetics - dentistry in Germany provides all types of dental services. German dentistry pays special attention to issues of prevention and preservation of dental health from an early age [6, 20]. In Germany, there are two systems of health insurance: mandatory and private (voluntary), respectively, there are two lists of dental services with estimated indicators of labor intensity and cost. The list of compulsory health insurance is contained in the Tariff Agreement (Bewertungsmaßstab fuer die vertragsaerztlichen Leistungen). The general list of dental services, which are paid from the personal funds of citizens or voluntary insurance, is given in the Regulations on tariffs for dentists (Gebuehrenordnung fuer Zahnaerzte - GOZ). Today, at the expense of compulsory health insurance (OMS), German hospital funds, a full spectrum of dental orthopedic care. The list includes 31 positions on prosthetics (not including other dental manipulations, for example, examination methods, control measures, anesthesia, periodontal examination, etc.) [6].

The main methods used in treatment in a German dental clinic are:

- stomatological prevention of diseases of the oral cavity;
- cosmetic dentistry;
- prosthetics (including the use of implants);
- use of bone tissue augmentation methods;
- computer three-dimensional radiography.

Germany, a country with traditionally strong state power, started developing and implementing public-private partnership projects relatively late. Only since 2002, the public-private partnership market has been actively developing there, providing increased efficiency in the use of budget funds, and allowing the creation of new objects of social, economic, environmental, and informational infrastructure.

Dentistry in Italy, as a professional specialization, exists within one of the general divisions of medicine. This explains the absence of a separate dental service in the country. According to statistics, out of 48 thousand doctors in this country, approximately 10 thousand specialists provide full-time dental care. In addition to

them, about 2 thousand dentists work part-time. The professional training of doctors in this specialization consists in completing 3-year courses after receiving a diploma in a general medical specialty. This is a mandatory condition for practicing dentists who have valid contracts in hospitals and other medical institutions belonging to the social security structure. The provision of dental care in Italy is mainly carried out on a paid basis at non-subsidized rates. From the funds of social insurance funds, patients are reimbursed:

- 1/3 of the cost of medical services for tooth extraction and caries treatment;
- 1/3 of the cost of dental filling medical services;
- 1/4 of the cost of dental prosthetics.

In the north of Italy, which is considered to be a more economically developed region of the country, the supply of dentists is higher than the national average. This is explained by the fact that in the center and north of the country, there is a much larger number of large cities with a population of 1 million or more. The phenomenon of economic development also determines higher tariffs for dental services, which are on average 20% higher in the north than in the south of the country. The first Italian experience of attracting private capital to provide services in the social sphere dates to 1923, when the first law on concessions was issued, and in the field of health care, the first projects started in 1998. Already in 1999, several projects were introduced to attract private investment in health care with the help of public-private partnerships for approximately 6 billion euros. This allowed Italy to take 3rd place in the world in terms of investment in health care after Great Britain and Canada [21]. In Italy, about 50 public-private partnership projects for large hospitals and homes for the elderly have already been signed or are in the preparatory stage. 30 of them refer to the creation of medical centers with 600 beds or more for a total of 3.5 billion euros [21]. Following Italian legislation, which does not conflict with EU legislation, public-private partnerships can be implemented in three forms:

- 1) concessions within the framework of a public initiative;
- 2) concessions within the framework of the private sector initiative;
- 3) concessions for service contracts that do not include large objects.

In Switzerland, the dental care system is similar to the German one. It includes health funds, voluntary health insurance, accident, and disability insurance. Hospital funds, in various cases determined by law, bear full or partial costs of providing dental orthopedic care. The public-private partnership in Switzerland has a clearly expressed focus - the satisfaction of the state interest

[22]. For certain categories of the population, the costs of necessary dental treatment are compensated (partially or completely) by social insurance. In Switzerland, in Basel, drinking water is fluoridated and pediatricians prescribe sodium fluoride tablets to children according to indications [22]. In France, industrial enterprises, municipalities, social security organizations, and various mutual funds (for example, funds organized by students or civil servants) have private or public dispensaries [9]. A patient insured in the social insurance system can choose a dentist and type of treatment. In addition to social security contributions, the French themselves pay 30% of the dentist's fee. Fees paid to dentists provide regular and comprehensive care to the entire population; groups that need special attention are not highlighted. The benefits for the private and public sectors from the use of the specified public-private partnership mechanisms are as follows:

- for the private sector: receiving a guaranteed income during the entire duration of the project for the provision of services ordered by the state, as well as expanding the boundaries of its activity;
- for the public sector: improvement of the «price-quality» ratio of the project implementation, compared to the traditional way of its implementation by the state (through a state order), as well as a reduction of the fiscal burden on the state budget due to the distribution of payments (if they are foreseen) for the implementation of the project to the private sector for a long period.

In France, public-private partnership projects are also successfully used in the practice of building health-care facilities. Thus, the project of the clinical center «Sud-Francillen» was transferred to a large construction and concession group in France. The contract was concluded as a result of a competitive struggle, during which Elqafé won the contract over its main competitors - other French companies, thanks to the existing experience of participating in the implementation of public-private partnership projects in France. The agreement on the creation of the project and the construction of the clinical center «Sud-Francillen» is an example that contributes to the partnership process as a whole. In Europe, as a result of joint activities, in the course of long discussions, the basis of the model was developed, which is called the system of balanced indicators. The approach to the management of dental organizations involves the selection of at least two fundamental problems: improvement of dental care itself; improvement of the dental care management system [23].

The improvement of dental care is inextricably linked with the introduction of new dental medical technologies, the improvement of professional training of the

medical staff of dental organizations, and the improvement of the quality of dental care, controlled through the system of standards in dentistry.

Improving the management system of dental care for the population relates to the introduction of advanced management technologies capable of providing the necessary information for analyzing the activities of a dental organization in four main aspects: «medical care», «personnel», «patients» and «finances». The choice of management decision is related to the choice of innovative medical technologies that meet medical, economic, and social criteria.

Investment models of joint participation of the state and the private sector in the field of health care have different forms. In some countries, the main emphasis is on investments in the creation of new infrastructure facilities, and in others - on improving the efficiency of existing ones [24-26].

From all the variety of public-private partnership models that exist in the world, the following main ones can be singled out [27]. Franchising - a private company enters a contract with a state or municipal customer for the management and operation of an existing healthcare facility. The private partner invests in the equipment of the health care facility, its equipment, and vehicles, ensuring the return on the invested investment and obtaining profit due to the efficient operation of the facilities [28-29].

No state in Europe fully provides the population with high-quality and modern dental care at the expense of the budget and is not able to allocate the necessary funds for modern dental equipment, technologies, and materials for the treatment (prevention) of dental diseases in the population.

The main features of European dental care include the availability and quality of dental care, the integration of dental and general health care services, the use of a team method of work in dental institutions, high-tech equipment in dental offices, and preventive work among the population. In the European dental service, two fundamental problems are highlighted: improvement of dental care itself as well as of the management system of dental care. The improvement of the actual dental medical care is developing along the path of state support of the dental care system; availability of several sources of funding for dental care; observance of human rights in the health care system; responsibility of the state (in any form) for dental assistance to socially vulnerable sections of the population. In conclusion, it can be stated that the global practice of providing dental care to the population has the experience of attracting patients to pay for dental services and a rich arsenal of organizational, economic, and legal

mechanisms for planning, regulating, and managing the activities of subjects of the dental services market. The essence of the above is reduced to the improvement of the dental care management system and, in our opinion, consists of a reasonable combination of administrative and professional management of the dental care organization.

CONCLUSIONS

The conducted scientific analysis of normative documents and domestic and foreign sources confirmed the expediency of reforming the dental service and substantiated the need to improve dental care and improve the dental care management system to increase medical, social, and economic efficiency.

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REVIEW ARTICLE

ANALYSIS OF THE WORLD'S BEST PRACTICES IN TRAINING PHARMACISTS FOR PROVIDING MENTAL HEALTH CARE TO PATIENTS

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ABSTRACT

The article analyzes and systematizes international documents and research results, which reveal the experience of implementing the best practices in mental health into the educational process of training pharmacists at various levels of education. Available foreign educational programs were analyzed in terms of their form and content. According to the results of the research, it was found out that the practice of professional training of pharmacists on mental health issues is quite widespread at the world level and is presented in various scientific publications. The results of their analysis also indicate different forms of training process organization.

KEY WORDS: mental health, students, pharmacy, pharmacies

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INTRODUCTION

According to WHO's world statistics (2023), mental and neurological manifestations account for 13% of the burden of disease and have a negative impact on the economy, increasing unemployment and health care costs. During conflicts, wars, and pandemics, the need for mental health care increases [1]. Consequently, there is a great demand for mental health services for the population of Ukraine related to the consequences of the COVID-19 pandemic and the war. According to statistical data of monitoring companies, about 70% [2] of the population of Ukraine has a mental health disorder, which requires a solution through the development of a sustainable system of mental health care to "bridge the gaps" [3] and provide access to medical/pharmaceutical care to the population in accordance with the Sustainable Development Goals. The issue of implementing the best foreign experience in providing pharmaceutical assistance in the field of selling medical products to patients with mental health disorders in the educational process of training pharmacists at various levels of education in connection with the expansion of the Roles of Good Pharmacy Practice is obtaining relevance. The need to create a professional environment for pharmacy pharmacists in the context of building a mental health care system is obvious. Thus, the implementation of the best world practices in the

domestic system of training pharmacists capable of providing pharmaceutical assistance at the appropriate level using modern approaches will allow maximizing of the professional potential and prevention of complications of diseases in a timely manner. Therefore, the organization of training of pharmacists in mental health issues is relevant at the time in all countries, especially in Ukraine.

MATERIALS AND METHODS

Scientific publications, international documents, websites of international organizations and of associations of pharmacists, official websites of educational institutions, educational portals and training centers, were used to achieve the goal of the research. Methods of systematization, analysis and generalization were applied.

REVIEW AND DISCUSSION

The results of the analysis of official international documents and scientific works showed that the organization of training of pharmacists to provide pharmaceutical care for the mental health of the population is relevant in many countries of the world, i.e.: Australia [4], the USA [5], Great Britain and Ireland [6], Canada [7], New Zealand [8], Granada [8], Sweden [9], Brazil [10] etc.

Results of analysis of publications in scientometric databases Web of Science, PubMed, Elsevier, etc. to find out the state of readiness of pharmacists to provide pharmaceutical assistance to the population on mental health issues, testify to the high interest of foreign scientists in the implementation of mental health issues in the training programs of pharmaceutical personnel at various levels of education, i.e.: bachelor's, master's, continuous professional development. Thus, in the scientific works of Australian scientists El-Den, S., Moles, R., Choong, H. J., & O'Reilly, C. (2020), the positive impact of the American MHFA mental health education program for students of various specialties was investigated [11]. Scholars pay special attention to the specialized MHFA mental health program for students of pharmaceutical faculties, which has been implemented in 25 countries around the world. The MHFA program is included into the mandatory, after which the assessment of mental health knowledge, willingness to help, the degree of stigmatization and the application of skills in practical activities is carried out. At the same time, scientists emphasize that for the objectivity of assessing the acquired knowledge and skills of students, assessment based on competencies should be used [12].

English scientists H.C. Gorton, J. Strawbridge, H. Macfarlane (2023) came to the same conclusion. According to the survey data among students of pharmaceutical faculties, it was found that the students understand the importance of psychiatric care and agree with the need to expand the role of pharmacists in the Good Pharmacy Practice regarding the provision of patient-oriented care. Based on these data, the scientists reached a unanimous opinion regarding the use of a complex (integrated) approach to the formation of knowledge and skills in mental health care [13].

A systematic review of the implementation of educational programs on mental health issues in the system of master's degree training and postgraduate education, conducted in accordance with all the requirements of the Cochrane community by researchers Carmen Crespo-Gonzalez, Sarah Dineen-Griffin, John Rae, Rodney A. Hill (2022) from Australia is of scientific interest to us. Scientists have proven that the quality of acquired skills and the confidence of pharmacists and pharmacy students in providing first aid for mental health positively influenced the further pharmaceutical practice of providing such services to patients. Australian scientists investigated the quality of knowledge and skills acquired by pharmacists and students of pharmaceutical faculties after completing training in mental health educational programs. The result of the clinical study was the proven effectiveness of educational programs on the mental health of the population in the long

term at the pre-diploma and post-diploma levels of education [14].

A review of the scientific literature conducted by scientists Mohammed Kanan Alshammari, Nawaf M. Alotaibi, Suroor Nasser Al Suroor from Saudi Arabia requires special attention [15]. Scientists divided the roles of pharmacists in mental health care into conventional, extended and advanced pharmaceutical services. Their conventional services include high-quality use of MPs in medical institutions, pharmaceutical support for prescriptions (explaining to the patient the methods and features of MP's application, etc.). The scientists included the following services of pharmacists for mental health care as extended: management of the range of MP's in the patient's home first-aid kit, joint prescribing of medicines by a doctor and a pharmacist (Australia); medical products use review services, specialized teams, which include pharmacy employees, primary and secondary care physicians, solving problems related to MP's use (Great Britain); management of drug therapy, application of clinical telemedicine (USA); drug review services (New Zealand). Scientists also draw attention to the problems faced by pharmacy employees, in particular: lack of time, low awareness of patients regarding the possibility of depression screening in general practice pharmacies. The problems of implementing first aid by pharmacists are important, which consist in patient education and adaptation of protocols for pharmacists to provide primary care in mental health issues [15].

Equally important are the issues of confidentiality of patient data and support from pharmacy owners regarding patients' mental health. The low level of knowledge of pharmacists regarding mental health issues requires the adjustment of educational programs and appropriate didactic approaches on the part of teachers. Researchers from Saudi Arabia argue that educational programs should adapt to education about the pharmacological properties of antipsychotic MPs, which are based on the principles of evidence-based medicine, as well as to focus on reducing the stigma of mental health and developing communication skills [15].

Ukrainian scientists L. Hala [16], Ja. Hrynkiv [17], O. Zalizka [18], H. Panfilova [19] paid considerable attention to issues related to the organization of pharmaceutical care in accordance with the requirements of Good Pharmacy Practice. The question of potential stressful factors in the practice of pharmacists was studied by V. Tolochko, I. Mishchenko, who paid considerable attention to the psychological training of pharmacists [20]. However, the problem of training pharmacists in mental health issues was not given appropriate attention.

Table I. Professional and educational and training programs in mental health (developed by the N.Bilousova)

Educational and training programs	Professional and educational programs	<p><i>Mental health as a component of master's programs</i></p> <ul style="list-style-type: none"> • 25 countries of the world (universities of Belgium, Great Britain, USA, Australia)
		<p><i>Postgraduate training programs (PhD) - postgraduate studies with a certificate/diploma</i></p> <ul style="list-style-type: none"> • Ability to work in the field of mental health policy, public health, scientific research Great Britain, Australia, USA
<p><i>Mental health as a clinical pharmacy course module</i></p> <ul style="list-style-type: none"> • mental health is represented by separate modules in clinical pharmacy programs • Bulgaria, Poland, Romania, Belgium, Estonia, Lithuania, the Netherlands, Jordan, Israel, Saudi Arabia 		
<p><i>Specialization of pharmacists in psychiatry followed by pharmacist-psychiatrist certification (2 years)</i></p> <ul style="list-style-type: none"> • training of pharmacists for consulting activities in pharmacies and providing pharmaceutical first aid • USA, Australia, Great Britain 		
<p><i>Continuous professional development courses for pharmacists</i> (Australia, USA, Great Britain, Bulgaria, Poland, Czech Republic, Romania, Belgium, Netherlands, Jordan, Saudi Arabia)</p> <ul style="list-style-type: none"> • Short-term courses (20 minutes - 2 hours) • Short-term training programs (8-12 hours) with three qualification levels 		
<p><i>Online courses at pre/post diploma levels for a wide range of participants</i></p> <ul style="list-style-type: none"> • public organizations, associations of pharmacists) on mental health • USA, Great Britain, Australia, Belgium, Ukraine 		
Educational and training centers	Educational and training programs	<p><i>Mental Health Instructor Courses followed by certification after completion of the Mental Health First Aid Program</i></p> <ul style="list-style-type: none"> • USA, Australia, Great Britain, Ukraine
		<p><i>Introductory programs on mental health issues</i></p> <ul style="list-style-type: none"> • EU countries, USA, Australia, Great Britain, Ukraine

Considering the stated topic of the research, it should be noted that the consequences of the COVID-19 pandemic, the bloody war in Ukraine, and the European integration processes make significant adjustments to the organization of professional training of pharmacists. In this context, the question of transformation of the mental health care system of Ukraine in relation to the program of actions to overcome the gaps in the field of mental health care (mhGAP) [3] is in the center of the WHO's attention [21].

In the developed recommendations of the WHO, in view of the transformational processes taking place in the health care system in Ukraine, attention is focused on the creation of high-quality educational medical and pharmaceutical programs for the training of pharmacists [22]; increasing the number of practical training of pharmaceutical personnel on a modular basis; professional regulation in the field of health care [23].

Supported by WHO, the International Pharmaceutical Federation FIP in the global call to action on pharmaceutical education emphasizes the transformation of the training of "a flexible and adaptable pharmaceutical workforce to maximize the application of their knowledge, skills, attitudes, behaviors and abilities as part of a multidisciplinary health care team". Particular attention is paid to identifying new areas of competence development, digital health and patient safety, improving interdisciplinary, interprofessional education and collaboration with key parties concerned [24].

In the same context, Resolution CM/Res(2020)3 of the Committee of Ministers of the European Commission on the provision of pharmaceutical care in the interests of patients and health care services is of scientific interest to us [25]. Thus, the document emphasizes interprofessional relationships between pharmacists, patients, primary care physicians and nurses in order to ensure

Table II. Comparative characteristics of educational programs for different users (developed by the N. Bilousova)

Mental health programs (for a wide range of participants)	Mental health programs (for pharmacists)
panic attacks; suicidal thoughts and behavior; non-suicidal damage; acute psychosis (hallucinations; overdose or withdrawal from alcohol or narcotic MPs); reactions to traumatic events; mental health problems; an action plan for providing primary care in the field of mental health; depression; anxiety; mental health crises (panic attacks, people after traumatic events, people with aggressive behavior)	diagnosis and classification of mental disorders (panic attacks; suicidal thoughts and behavior; non-suicidal self-harm; psychosis; use of psychoactive substances; severe consequences of alcohol or other drug use; depression; anxiety; stress; traumatic events; post-traumatic stress disorders; severe psychotic states; aggressive behaviour; dementia and geriatric disorders; HIV and depression; adolescent mental health; schizophrenia; dementia; eating disorders; learning disabilities; perinatal mental health, etc.); key concepts and terminology; the latest methods of treatment of mental disorders based on evidence medicine; evidence-based pharmacological and psychological therapy; secondary psychiatric services; the effectiveness of taking drugs for the treatment of long-term mental disorders; repeated monitoring of patients in the short-term; forensic medical examination; management of stressful situations; additional support for mental health and well-being; bullying and conflicts at the workplace; increasing stability in the pharmacy team; advice on the use of fluoxetine and peculiarities of the use of clozapine; improvement of adherence to treatment of patients with depression; mental well-being of patients; training of patients and other individuals; sexual harassment in pharmaceutical practice; providing leadership in the health care system and public policy on improving the health of people with mental illness

effective and safe use of MPs. Therefore, to solve this issue, it is proposed to build such a health care system, where pharmacists and primary care doctors will act as a single link and solve part of the pharmaceutical care issues in order to implement all the clinical skills acquired in the training process. In turn, for the purpose of control, quality indicators of the provision of pharmaceutical care have been developed [26].

Our attention is drawn to a practicum for pharmacists on providing psychological first aid and psychosocial support to diverse populations, developed by Cory P. Coffee (2023) of the Ohio State University College of Pharmacy with the support of the Ohio State Pharmacists Association (USA). According to his recommendations, population groups are divided according to various states of well-being proposed by the International Pharmaceutical Federation (Fig. 1). The researcher proposes to introduce protocols for providing assistance to patients in the field of mental health, developed on the basis of evidence-based medicine. Cory P. Coffee pays special attention to the role of pharmacists in providing psychosocial support and psychological assistance for various mental health conditions [27].

Therefore, foreign programs on mental health issues for a wide range of users, including pharmacists, developed on the basis of competencies, have proven effectiveness in the long-term perspective of formal and informal education (master's educational and professional programs, thematic improvement courses for

pharmacists, professional seminars, trainings, etc.) [29].

This approach is important in the provision of pharmaceutical patient-oriented care in the field of selling MPs for the prevention and treatment of patients with mental health disorders at the levels of mental well-being, mild and moderate distress and mental health disorders. We analyzed available mental health educational programs and online resources aimed at raising awareness of this issue. Based on the results of the research, eight different most common areas of professional educational and training programs for training pharmacists in mental health issues with the use of modern educational technologies, have been identified (Table I).

The American MHFA distance course [30] is of specific scientific interest. The course lasts 8 hours and is intended for a wide range of participants. The pharmacist course curriculum provides an in-depth study of mental health issues and includes the following primary care modules: depression and mood disorders; anxiety disorders; panic attacks; traumatic events; psychosis; disorders related to the use of psychoactive substances; severe mental conditions.

An Australian blended (online/offline) mental health primary care course has developed a competency framework for pharmacists to acquire upon completion of the course. The course curriculum provides an in-depth study of mental health issues for pharmacists and includes four three-hour sessions with presentations,



Fig. 1. States of mental health according to [28].

videos, practical sessions, group work and role-playing. The course consists of two components: interactive e-learning for self-study (5-7 hours); instructor-led video conference or classroom training (2 - 2.5 hours). The content of the training course includes the following topics: mental health problems; an action plan for providing primary care in the field of mental health; depression; anxiety problems; panic attacks; psychosis; serious traumatic events; problems with the use of alcohol and narcotic MPs; severe mental conditions; mental health crises [31].

It is worth mentioning, that professional educational programs for pharmacists are implemented in various universities around the world. These include the University of Manchester, College of Pharmacy and Mental Health (Great Britain) [32], which have implemented both short courses in mental health and longer educational programs to train pharmacists in postgraduate continuous professional development (CPD). There is also a focus on longer-term educational programs to integrate pharmacists into mental health to intervene in patient care to improve medication and treatment adherence for depressed patients [33].

It should be noted that Queen's College (Great Britain) [32] jointly with Birzeit University (Palestine) [34] offer 4-week training courses for pharmacists in the process of continuous professional development in mental health issues, developed on the basis of the National Competence Frameworks of these countries. The intervention strategies in the mental health of citizens, mental health practice, methods of mental health research in conditions of war and military conflicts, consultations for patients with mental health disorders, which are considered in the training process, are of special interest.

Our attention was drawn to the integration program with the expansion of Roles of Pharmacists in Mental Health offered by the University of Bradford (Great Britain). It is developed on the basis of the National Competence Framework. The curriculum of the course involves 12 months of instructional training and includes 10 modules: assessment of instructional training needs; work in community groups for mental health care; mood disorders; audit and assessment of services; psychosis and schizophrenia; care for the elderly; education of patients and other persons; misuse of psychoactive substances; complex and multiple disorders; quality improvement and research [35].

In our opinion, the two-year American BPS Board Certified Psychiatric Pharmacist (BCPP) specialization program for pharmacists in psychiatry, developed on a competency basis, requires special attention. The program includes training modules for developing, implementing, monitoring and changing patient treatment plans; education of patients, medical professionals and other interested parties; providing leadership in health care and public policy to improve the health of people with mental illness. The main vectors of the development of specialization of pharmacists in mental health are person-centered care; transition of evidence into practice and education; health policy, advocacy and mental health practice management.

The results of a comparative analysis of various professional education and training programs for pharmacists allow us to summarize that they include both topics from training courses for a wide range of participants with developed guidelines for providing first aid for the community in the field of mental health and in-depth training on mental health, which is based on evidence (Table II).

It should be noted that distance learning is now widely used, including for educational programs on mental health. Online courses on mental health issues can currently be found on the websites of universities or pharmaceutical societies (public organizations). Such training programs are of short duration (from 20 minutes to 2 hours) and are offered by various professional communities and educational platforms (for example, the community of pharmacists of Great Britain "C+D Community" [36], Future Learn [37], MH4U (Ukraine) [38], Prometheus (Ukraine) [39], Academy of the National Health Service of Ukraine [40]) etc. The educational portal Future Learn offers 4-week courses on mental health issues for pharmacists, which are based on the principles of evidence-based medicine, and the Ukrainian platform of the MH4U project offers short-term courses on mental health issues for a wide range of users. Short-term online courses "Mental Health Self-help for Veterans"; "Traumatic Experience and PTSD" for psychotherapists, etc. are offered by the educational web-site Coursera. The Academy of the National Health Service of Ukraine (NHSU) offers an online course for family medicine doctors "Therapy of common mental disorders at the primary level of medical care by using the mhGAP guidelines" with the support of WHO, the Ministry of Health of Ukraine and the NHSU.

Therefore, summarizing the results of the analysis of the best global practices, we can conclude that the practice of professional training of pharmacists in mental health issues is quite widespread at the world level and is presented in various scientific publications. The results of the conducted analysis indicate different forms of organization of this training. Therefore, in our opinion, it is quite logical to introduce training programs on the mental health of the population into the practice of professional training of Ukrainian pharmacists both at the master's level and in the system of continuous professional development.

CONCLUSIONS

1. The international experience of professional training of pharmacists in mental health issues is summarized for further implementation in Ukrainian professional and educational programs of training pharmacists during lifelong learning.

2. It was found that foreign educational programs on mental health issues were developed on the basis of modular and competency-based approaches. The most common form of education is informal education (specialization of pharmacists in mental health issues, thematic improvement courses for pharmacists, professional seminars, trainings, schools in mental health issues, etc.).
3. Foreign educational programs on mental health issues are offered by institutions of higher education, pharmaceutical societies, public organizations, educational Internet portals, which indicates a high interest of society in this issue.
4. The content analysis of available educational programs for pharmacists in terms of structure and content showed eight different, most widespread areas of professional-educational and educational-training programs for training pharmacists on mental health issues using modern educational technologies.

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REVIEW ARTICLE

THE RIGHT TO HEALTH CARE IN THE CONTEXT OF PUBLIC AND PRIVATE INTERESTS

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ABSTRACT

The aim: To review of existing approaches regarding the ratio of private and public interests in the content of the right to health care.

Materials and methods: In this paper, a systemic approach was used, which made it possible to analyze public-law and private-law interests as part of the content of the right to health care in their systemic unity. The application of the comparative research method and the dialectical method made it possible to investigate the dynamic relationship between public legal and private legal interests as part of the right to health care.

Conclusions: In the context of the analysis of the content of the right to health care, it was determined that private and public interests find their form in the institutions of individual and public health. In conditions of economic stability and the absence of threats to the realization of the right to health care, the state of realization of the right to individual health and public health can be described by the categories of «binary» or «synergy», when public health and individual health strengthen each other one. Private and public interests in the content of the right to health care can be in a state of conflict in conditions of limited resources. Taking into account the need to legitimately regulate the conflict between private and public interests in the content of the right to health care, states establish legal norms, which we defined as «axiological collisions».

KEY WORDS: right to health care, public interest, private interest, health care system

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INTRODUCTION

In our multifaceted world, the right to health care is considered one of the fundamental legal values, as a prerequisite for the existence and development of a person, a key to the functioning of an efficient economy and the progressive development of society as a whole. Despite the long historical path of humanity to the recognition of this right and its consolidation in national legal systems and acts of international law, the debate about the content of the right to health care, its «polyvalence» from the point of view of private and public interests, has not lost its relevance at the present time.

As we know, since the days of Roman law, the main criterion for distinguishing between private and public law – legal interest – has been considered the most important «engine» of human actions, the motive for any legally significant behavior. Currently, the division of law into private and public is considered one of the features of continental European (Romano-Germanic Legal System) law [1]; the legal systems of many civilized countries are based on the principle of dividing law into private and public (Germany, France, Italy, Spain, and others) [2].

A wide range of researchers have paid a lot of attention to the study of various aspects of legal interest,

the dichotomy of public and private interests in various doctrines and legal systems [3-7]. As should be clear by now, the position one takes on the distinction between private law and public law is both theoretically and practically important [7]. From both a theoretical and a practical point of view, it is important to trace the connection between legal interests and the formation of conceptual approaches to the right to health care.

After the World War II the most important trend in the development of national legal systems and international law was the desire to create a global «legal infrastructure» of human rights. The mutual process of development of global human rights law and the implementation of the concept of human rights in national legal systems took place against the background of a powerful scientific discourse, the issues of which were related to the justification of the universal nature of human rights against determinism, the search for a certain balance of public and private interests in determining the content of human rights. Joseph Raz describes the specified problems regarding the right to health care in categories «compromise between concern for health and the pursuit of other values» [8].

Even a superficial overview of the existing multifaceted approaches to the concept of the right to health

care allows us to identify a certain «conflict potential» in the content of this right: 1) how widely we are ready to understand the content of this right and the interests related to it, in order to provide it with effective guarantees implementation; 2) how widely we are ready to recognize the subject field of this right, realizing that this field may overlap with other individual rights and interests (for example, with the right to choose one's behavior, including the self-destructive one); 3) to what extent we are ready to protect this individual right, including in the situation of its conflict with certain public interests and values, primarily with the interests of public health. The solutions to these issues lie at the intersection of philosophy, politics, bioethics, law in general and legal axiology in particular.

The above mentioned determines the relevance of right to health care research from the point of view of the ratio of private and public interests in its content.

THE AIM

The aim is to review of existing approaches regarding the ratio of private and public interests in the content of the right to health care.

MATERIALS AND METHODS

In this paper, a systemic approach was used, which made it possible to analyze public-law and private-law interests as part of the content of the right to health care in their systemic unity. The research process was carried out on the basis of the principles of historicism, objectivity, complexity and reasonableness, which created a methodological basis for the analysis of the research subject. The application of the dialectical method made it possible to investigate the dynamic relationship between public legal and private legal interests as part of the right to health care. In addition, the research used such methods as an abstract logical method for theoretical generalization and conclusions, a method of analyzes and synthesis for the determination of the relationship between public and private interests. The conclusions obtained in the course of the work would not be sufficiently substantiated without the use of the comparative research method.

REVIEW AND DISCUSSION

The criterion of the nature of the dominant interest, allows to distinguish public law, which protects the general, state, public interests, public goods, important not for the individual but for the society, country as a whole. Instead, in private law, the personality interests of the

individual (persons) are dominant [9]. In the context of the analysis of the right to health care, it is appropriate to distinguish between individual health and public health, which, in our opinion, are institutionalized forms of private and public interests in the field of health care.

As we know, Constitution of the World Health Organization (WHO), signed at New York on 22 July 1946, declares the value of the individual right to health care (one of the fundamental rights of every human being), as well as the value of the world legal order – «the health of all peoples». As stated in the WHO Constitution: «Governments have a responsibility for the health of their peoples which can be fulfilled only by the provision of adequate health and social measures» [10]. That implied the provision of basic preventive and curative medical services and the provision of medicines, the creation of the necessary system of medical institutions located in places accessible to the people, as well as the availability of qualified medical specialists. Thus, the approach defined by the WHO emphasized the associative relationship between the human right and the obligation of the states in the field of health care.

The human right to health appears in Article 12(1) of the 1966 International Covenant on Economic, Social and Cultural Rights (ICESCR): The States Parties to the present Covenant recognize the right of everyone to the enjoyment of the highest attainable standard of physical and mental health [11].

Attainability connotes duties being relative to the economic, social and political circumstances of different countries [8]. Thus, the widest list of restrictions related to the practical implementation of the individual right to health care lies in the plane of the real capabilities of each state to ensure the implementation of the researched right. It will not be news to claim that each sovereign state is looking for its own way of implementing social policy and achieving a balance of private and public interests. In our opinion, the amount of social obligations guaranteed by the state in the field of health care is directly proportional to the recognized public interests in this field. Taking this into account, each state also establishes certain restrictions for the exercise of the right to health care.

For example, the Constitution of Ukraine (articles 35, 39) defines restrictions on the exercise of human rights may be established «in accordance with the law and only in the interests of national security and public order, with the purpose of preventing disturbances or crimes, protecting the health of the population, or protecting the rights and freedoms of other persons» [12]. It is not difficult to notice that public interests are really «weighty» limitations of individual rights (private interest).

The development of the theoretical and law enforcement potential of the concept of human rights, which took place during the second half of the 20th century, led to a misleading global confidence that «The international community must treat human rights on a global, equitable and equal basis» [13]. It was in such a paradigm – equal access to versatile legal opportunities – that scientific research of right to health care were mostly carried out.

It seems that we are unlikely to find reliable information about the date in historical time when the justification and implementation of the concepts of the individual right to health care began. «The right to health ... is a product of the Industrial Revolution in 19th-century Britain, in circumstances analogous to those in less developed countries today» [14].

As we know, the individual right to health care is relatively conditionally classified as a second generation of human rights (socio-economic and cultural rights), which gained recognition and consolidation during the days of socialist revolutions. Traditionally, the theory of the division of human rights, which was developed by the French lawyer Karel Vasak, is used to classify human rights. The basis of this concept is the generational approach, namely, the division of rights into three generations [15].

Since the recognition and proclamation of the individual right to health care, many conceptual approaches to understanding health and the content of the right to health care have been developed. «...it is well known that health is subject to conflicting meanings, from narrow biomedical definitions based on the statistically normal functioning of the human organism, through to very broad accounts of positive well-being and human flourishing, such as the World Health Organization's concept of «complete physical, mental and social well-being» [16]. There is not a linear spectrum of concepts of health. Rather, there is a plurality of accounts, with a notable added dimension when we contrast individual and population-level understandings [17].

It can be argued that concepts of public health are equally multifaceted. As Encyclopedia Britannica states, «Are view of the historical development of public health, which began in ancient times, emphasize show various public health concepts have evolved» [18]. It is important to emphasize that in the scientific discourse, the concept of «public health» is very often identified with the concept of «population health», using these terms as synonyms.

The field of public health is closely related to population health, so much so that the two terms are sometimes used interchangeably. A key distinction: Population health tends to focus on a narrow group,

usually determined by geographic boundaries. Public health often addresses larger communities, including those determined not by geography but by race, gender, immigration status, disability level, or other factors [19]. If these definitions are causing more confusion than clarity, take a step back and consider that «public» and «population» are synonyms and in most cases, so too are «public health» and «population health» [20].

Population health is a relatively new term that has not yet been precisely defined. We propose that the definition be «the health outcomes of a group of individuals, including the distribution of such outcomes within the group,» and we argue that the field of population health includes health outcomes, patterns of health determinants, and policies and interventions that link these two [21].

«Population health is an opportunity for health care systems, agencies, and organizations to work together in order to improve the health outcomes of the communities they serve». «...at the core of public health lies the principle of social justice: making sure that we are providing people the right to be healthy and to live in conditions that will support their health». Broadly thinking about it, you could say public health is about what we're doing as a society and population health is about what a system is doing for their community [22]. For the purpose of our research, it is important to emphasize that the terms «public health» and «population health» (regardless of their substantive differences) appeal to public interests, the carriers of which are certain communities – social groups, the population of a certain state or, even humanity as a whole.

These global public interests, the subject of which is humanity as a whole, are based on universal values (for example, the value of public health is universally recognized). That is, the recognition of such a large-scale public interest is based on the presumption that this interest corresponds to the will of the world community and concerns every member of this community. But can it be argued that the public interest of the highest attainable level of health is the sum of the private interests of all people?

«... the health right cannot entail a set of individual contracts to ensure a healthy state for each person. Instead, a societal contract is involved. The societal commitment necessarily entails equity between groups as a fundamental principle» [14].

This recognized fundamental principle of social justice is constantly tested by time and law enforcement practice. Arguably, the strength of the general consensus on «highest attainable health» is maximal precisely at the theoretical level that forms the center of this vicious circle. The further from this center, the

more tests stand on the way to achieving the specified formula – such tests are related to cultural characteristics (in contrast to the universal, impeccable value of the right to health care), private interests, moral and ethical choices.

In practically seeking to make health public, ethical practice requires three important considerations to remain in view. First, the particular public health goal requires a clear ethical mandate. Second, the means used to reach that goal needs to be justified; we cannot just defend a policy or intervention because its outcomes are well-intended. And finally, we must not lose sight of the fact that our public health ethics must ultimately sit within a wholesale public and social ethics [16].

Presumably to smooth over this immanent conflict (between declaration and enforcement practice, between public and private interests), a significant number of researchers insist on the integration of «individual health» and «public health» efforts, arguing that this is a win-win situation: «Public health needs clinical partners, and the path to meeting this need is through integrated training. Medicine needs the power of population health» [23]; «Public health and medicine approach the challenge of health and health care from distinct, complementary perspectives» [24].

The WMA insists that public health should not be seen in isolation, as it is intrinsically linked to individual health, health care and medical care. The WMA advocates for the development of integrated health care systems in which both public health and individual health can be addressed [25].

Arah OA. argues that neither individual nor population health is *identifiable* or even definable without *informative contextualization* within the other [26].

Researchers point to the objectively existing process of convergence of private and public interests. «...the dynamics of public and private interests currently show a tendency towards convergence, mutual penetration. The interaction of public and private law reflects the dynamic balance of the interests of political forces, the state system, management mechanisms, the degree of freedom and independence of citizens in the modern world, etc.» [27]. The use of the term «binary» to denote actions that are carried out «simultaneously in the interests of the whole society and in the interests of an individual seems to be successful» [28].

Realization of public interests is often a condition for realization of private interests. The opposite situation is also possible, when the realization of a private interest leads to the realization of a public interest [29], «...any private interest can be summed up, considered, presented as a public interest, because a separate, private interest is very often important for the social whole.

And vice versa: public interest without bringing it to any separate private interest becomes meaningless» [29].

Borrowing terminology from epidemiologic methodology, Arah OA. classifies the individual-versus-population health relationship into four categories [26]:

1. Immune: individual health remains good irrespective of the population health or context.
2. Causative: individual health is boosted in favorable population health or context.
3. Preventive: individual health is compromised when population health or context is unfavorable.
4. Doomed: individual health is compromised irrespective of the population health or context.

In our opinion, the dialectic of public-law and private-law interests is manifested in the fact that they can strengthen each other if they are synergistic (aimed at the same goal), or weaken each other if they are in conflict. Conflicts between public and private interests are activated in situations of choice in conditions of limited resources. Such resources can be all kinds of social and individual benefits, access to which directly or indirectly affects the real state of the realization of the right to health care (health care facilities, qualified doctors, medicines, medical technologies, information on effective clinical treatment protocols etc).

Trying to substantiate the formula of coexistence and mutual limitation of private and public interests, we will not find universal legal norms that determine the legitimate potential perspective of limiting public interests by private ones. At the same time, the limitation of private interests by public interests has a systemic nature, subject to compliance with certain legal conditions (determination of grounds, terms and elimination of collisions). «We define norm collisions as instances in which actors claim that two or more norms provide conflicting or incompatible expectations about appropriate behaviour in a specific situation» [30].

As we know, legal norm collisions serve as certain guideposts in situations of choosing between different legal prescriptions and eliminate legal uncertainty. Such norm collisions are used to regulate the choice between private and public interests. In our opinion, it will be correct to define them as «axiological collisions». In any case, the legislator must take responsibility for eliminating the axiological conflict on the basis of a certain moral choice, which is positioned for society as fair. «A system that distributes healthcare unevenly, on the basis of any determining factor other than necessity, raises numerous questions about how ethical that system is. In a society where disparity in the level of care or access to care exists, inevitably there will be individuals who fail to receive the care for which they desperately need» [31].

This is exactly the situation – the impossibility of access to medical care as a component of the individual right to health care – which has arisen as a result of the spread of the global pandemic for a significant number of people in different countries of the world. Presuming that the right to health care of an individual is the basis for realizing the right to health care for society as a whole, we lived in love with the «symbol of faith» – «the claim that all people alive today have the same human rights» [8]. Since the success of mass vaccination convinced us to look leniently at the processes of the spread of infectious diseases, we were sure that the epidemic well-being of society does not threaten the rights of an individual, which means that the rights of an individual can not in any case be sacrificed for the common good. The global pandemic – among other impacts on society – has actualized the conflict between public-law and private-law interests, the values of the individual right to health care and public health, as old as the world itself, and forced us to once again rethink their relationship.

CONCLUSIONS

The conceptual approach to the content of the right to health care, recognized at the global level (UN, WHO) and at the level of national legal systems, carries the ambivalent potential of the value of health in its individual and social dimensions. In the context of the analysis of the content of the right to health care, it was determined that private and public interests find their form in the institutions of individual and public health. In developed countries with socially-oriented models of health care systems, the correlation between the recognized scope of the right to health care and the positive obligations of the state is direct. In the con-

ditions of different political regimes, the fact that the right to health care includes private and public interests can be denied (when public interests are absolutized in totalitarian states), or can be used as a tool of political manipulation. Denying or ignoring private interests can cause the deformation of the entire legal system of society, lead to totalitarianism and nationalization of all social life. In this case, the value of individual health is leveled, and the right to individual health is absorbed by the right to public health.

In conditions of economic stability and the absence of threats to the realization of the right to health care, the state of realization of the right to individual health and public health can be described by the categories of «binary» or «synergy», when public health and individual health strengthen each other one. Private and public interests in the content of the right to health care can be in a state of conflict in conditions of limited resources, which are all kinds of social and individual goods, access to which directly or indirectly affects the real state of the realization of the right to health care.

Taking into account the need to legitimately regulate the conflict between private and public interests in the content of the right to health care, states establish legal norms, which we defined as «axiological collisions». In our opinion, «axiological collisions» should indicate the legal conditions and reasons, the range of subjects to which they apply, as well as be based on the principles of the rule of law, humanism, integrity, and comprehensiveness of the right to health care.

The theoretical and practical problems considered in our review article require a continuation of the scientific discussion in order to further justify the formula of the ratio of public and private interests in the content of the right to health care without threat to each of these components.

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MODERN TRENDS AND PERSPECTIVES OF THE DEVELOPMENT OF ADHESIVE DENTISTRY. INNOVATIVE TECHNIQUES FOR THE APPLICATION OF ADHESIVE SYSTEMS

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ABSTRACT

The aim: To study the latest approaches to optimizing the composition and application protocols of modern adhesive systems, which are used during the restoration of defects in hard dental tissues with restorative materials.

Materials and methods: Thirty articles published between January 1, 2020 and February 1, 2023 in the scientific databases PubMed, Scopus, and Google Scholar were selected. The selected scientific works contained the results of laboratory studies, systematic reviews, meta-analyses of the physical and mechanical characteristics of adhesive systems with a modified composition or application protocols different from the instructions of the manufacturing companies.

Conclusions: The most promising directions for improving adhesive systems are modifications of the composition and protocols of their use with the aim of deactivating matrix metalloproteinases, improving the structure of the hybrid layer due to the creation of a three-dimensional mesh of collagen fibres with optimal properties, the introduction of antimicrobial agents to slow down the growth of bacterial colonies along the line of the adhesive joint. The available research results of modified adhesive systems are often contradictory, which determines the need to develop standardized test methods to obtain more reliable indicators of their physical, mechanical and biological properties. In some cases, the consequences of non-compliance with the recommendations of the manufacturing companies are a significant deterioration of the characteristics of the hybrid layer, adhesive strength, marginal fit, which, in turn, explains the need for further search for an optimized composition and techniques for applying bonding agents to improve the prognosis of restorative treatment.

KEY WORDS: adhesive systems, adhesion to tooth dentin, restorative materials, modified composition, adhesive fixation protocols

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INTRODUCTION

The adhesive ability of some dental materials to the hard tissues of teeth is of great importance in many fields of modern dentistry, in particular for direct photocomposite restorations, tooth splinting, fixation of orthopaedic and orthodontic structures, etc. [1]. To ensure a long-term and reliable connection of heterogeneous materials and hard tissues of the teeth, adhesive systems (hereafter – AS) have been used for a long time, the development and improvement of which was determined by the desire to invent a material that is optimal in terms of its physical, mechanical and biological characteristics, capable of solving most clinical tasks of dentists.

The cause of a large number of complications in restorative treatment is considered to be imperfect adhesion of restorative materials to the dentin of teeth. The presence of a constant flow of dental fluid in the dentinal tubules, the activation of enzymes that destroy the hybrid layer, the high sensitivity of the adhesive

preparation technique to the skills of the operator lead to the occurrence of postoperative sensitivity, secondary caries, pulp inflammation and loss of retention of restorations [2]. The introduction into clinical practice of AS of the VII and VIII generations significantly simplified the restoration of teeth due to the reduction of the number of stages of adhesive preparation, on the other hand, the reliability of such restorations in the long term of operation is considered controversial [3]. In contrast to the latest AS, the “gold standard” in restorative dentistry is called the V generation AS, which are used according to the protocol of etch-and-rinse. These ASs have gained considerable popularity due to the proven high strength of the adhesive bond between restorations and tooth tissues during significant periods of observation. However, the application of such AS using the specified technique often causes postoperative sensitivity and inflammation of the dental pulp [3].

Thus, the existing AS have certain disadvantages, which explains the need to optimize their composition

and application protocols. The search and implementation of improved approaches in adhesive dentistry will increase the clinical effectiveness of direct and indirect restorations and improve the prognosis of rehabilitation of patients with lesions of hard dental tissues.

THE AIM

The aim is to study the latest approaches to optimizing the composition and application protocols of modern adhesive systems, which are used during the restoration of defects in hard dental tissues with restorative materials.

MATERIALS AND METHODS

Thirty articles published between January 1, 2020 and February 1, 2023 in the scientific databases PubMed, Scopus and Google Scholar were selected for the purpose of the study. The following keywords were used for the search: "adhesive systems", "adhesion to tooth dentin", "restorative materials", "modified composition", "adhesive fixation protocols". The selected works contained the results of laboratory studies, systematic reviews, meta-analyses of the physical and mechanical characteristics of AS with a modified composition or application protocols different from the instructions of the manufacturing companies.

REVIEW AND DISCUSSION

Several ways of increasing the strength and durability of the adhesive bond for V and VII generation AS are proposed, in particular by changing their application protocols. In a meta-analysis of 68 articles, which reported on the results of laboratory studies of the microtensile strength of dentin samples of extracted teeth with photocomposite materials fixed on their surfaces, it was demonstrated that under identical conditions, the indicators of the samples in which AS was applied with the use of additional manipulations reliably exceed the corresponding indicators of the samples made according to the instructions of the manufacturing companies [4]. For V generation AS, such manipulations were an increase in exposure time ($p < 0.001$), the use of electrophoresis ($p < 0.001$), application of an additional layer of AS ($p = 0.05$), active application of etching gel ($p = 0.02$) and adhesive ($p < 0.001$). Under the condition of using VII generation AS, the same additional means also improved the adhesive strength: increased application time ($p = 0.001$), use of electrophoresis ($p < 0.001$), double application of AC ($p < 0.001$), active application of the etching gel ($p = 0.01$) and adhesive ($p < 0.001$).

Therefore, by applying simple additional manipulations during adhesive preparation, it is possible to increase the strength of the bond between the photocomposite and tooth dentin in laboratory conditions, which requires further study in clinical studies.

The constant movement of dental fluid through the dentinal tubules in the direction from the pulp to the tooth surface causes difficulties in creating a reliable bond between the restorative material and the dentin during adhesive preparation and contributes to the hydrolytic destruction of the hybrid layer. By means of scanning electron microscopy, it was proved that the effect of iontophoresis on the tooth during the application of AS causes a short pause in the simulated movement of dental fluid, increasing the infiltration of AS in the dentinal tubules, both when applying adhesives using the total etching and self-etching techniques. The maximum depth of the formation of adhesive strands in the dentinal tubules for the control groups of the V and VII generations AS reached the level of 100 μm and 8 μm respectively, while the similar indicators for the same AS with the use of iontophoresis were significantly higher and amounted to 170 μm and 70 μm [5].

In order to increase the physical and mechanical properties of AS and bring them closer to natural human dentin, the addition of nanofiller (in particular, hydroxyapatite crystals) to the AS composition is proposed. When studying indicators of microhardness and quality of polymerization, higher values were obtained precisely in samples formed with the help of modified AS compared to the traditional V generation AS. The paper emphasizes the importance of fixed dimensions of the filler (20x20x50 nm) for creating a biocompatible hybrid layer with a high degree of its integration in dentin and, accordingly, achieving optimal characteristics of AS [6].

Another work proved the effectiveness of using biomimetic nanohydroxyapatite to increase the strength of the bond between the composite and dentin. Dentin samples of extracted teeth, the surface of which was treated with nanohydroxyapatite, after thermocycling demonstrated significantly ($p < 0.05$) higher dentin microhardness (46.44 ± 1.86 MPa) and the maximum strength of fixation to the restoration material (14.41 ± 1.87 MPa) in contrast to the same indicators of the control group with the traditional AS application protocol (38.51 ± 2.37 MPa and 7.20 ± 1.10 MPa, respectively). Similar results can be explained by the phenomenon of remineralization of dentin due to the formation of calcium and phosphate ion deposits, provided by the presence of nanohydroxyapatite [7].

In a similar way, the mechanical properties of the VII generation AS, modified with a solution of β -tricalcium phosphate of different concentrations, were improved.

In this work, higher indicators of adhesive strength were obtained – 27.75 ± 3.15 MPa and 30.50 ± 3.25 MPa for 5% and 10% solutions of the doped AS, on the other hand, the average strength value for the samples of the control group was found at the level of 24.7 ± 3.64 MPa [8].

Reliable adhesion of restorative materials is ensured primarily by the creation of a hybrid layer on the surface of the prepared dentine, consisting of collagen fibers impregnated with AS. During the operation of restorations, AS gradually lose their properties due to permanent cyclic masticatory load, the catalytic action of dentin enzymes, saliva and bacteria, significant temperature fluctuations. Enzymes released after conditioning of mineralized dentin, in particular matrix metalloproteinases-2, 8 and 9 (hereafter – MMP), activate the degradation of collagen fibres and reduce the quality of the hybrid layer, which in turn reduces the strength of the adhesive bond [3, 9, 10].

To limit the influence of these enzymes, it is proposed to use high-molecular aminosaccharides, for example, chitosan, the compounds of which reduce the activity of MMP, create additional cross-links between collagen fibres, demonstrate odontotropic properties, improve the physical and mechanical characteristics of AS, and have an antimicrobial effect [11, 12]. In a comparative study of the bond strength of V and VII generations AS, used in accordance with the manufacturers' recommendations or with prior application of a 2.5% chitosan solution on the surface of the dentin for 1 minute, it was established that 24 hours after the restoration of hard tissue defects of the extracted tooth samples according to the proposed protocol, the maximum tensile strength values, on average, by 5.77 MPa and 5.87 MPa exceeded the values of the samples produced by the classical techniques of V and VII generations AS, respectively. After 6 months, the same indicators were already 9.06 MPa and 5.33 MPa higher for similar groups of samples [11].

Greater effectiveness of chitosan is proven by the results of a similar study of adhesive strength by the tensile method of samples of intact (group I) and demineralized (group II) dentin. Under the condition of applying the same universal AS for both groups using the self-etching technique and after 6 months of artificial aging, the average strength indicators were 33.39 ± 9.92 MPa and 13.93 ± 5.34 MPa, respectively. At the same time, in the control groups of samples, the above-mentioned indicators were lower and amounted to 32.20 ± 5.85 MPa for intact substrate and 9.74 ± 4.90 MPa for demineralized one [13].

An approach to use the positive properties of chitosan by adding its nanoparticles directly to the AS composition is proposed in order to increase the number of ionic bonds between the chitosan molecules themselves, AS

monomers and the collagen network of dentin, which allows creating a strong three-dimensional structure capable of withstanding significant mechanical loads and the influence of the dentin enzymes [14]. It is also possible to introduce chitosan already into the structure of the restorative photocomposite material to obtain a homogeneous mixture. The reliability of the marginal adhesion of the photocomposite to the tooth tissues was studied on the restored samples by recording the depth of penetration of dyes at the dentin-restoration boundary. Thus, it was found that the marginal adhesion of the chitosan-modified photocomposite (the average depth of dye penetration was 0.01204 ± 0.00281 nm for V generation AS and 0.01182 ± 0.00311 nm for VII generation AS) was better, than in samples where defects were restored with the same photocomposite material without additional components (0.01834 ± 0.00181 nm for V generation AS and 0.01294 ± 0.00219 nm for VII generation AS). An important aspect is the non-significant ($p > 0.05$) differences between the indicators of the studied and control groups of samples in which self-etching AS was used [12].

Other MMP inhibitors include a 2% solution of chlorhexidine bigluconate (CH), a 17% solution of ethylenediaminetetraacetate (EDTA) and a 2% solution of doxycycline (DO), which are also applied to the surface of the dentin after conditioning and before applying AS [15]. In the study of the strength of the adhesive bond by the shear method with the use of V generation AS, immediately after the production of the samples, the average indicators in the studied and control groups did not differ significantly ($p > 0.05$). The value of the average strength for the samples treated with CH was 21.60 ± 5.73 MPa, with EDTA was 19.50 ± 3.44 MPa, with DO was 19.80 ± 3.89 MPa, only with AS was 19.20 ± 2.55 MPa. After 6 months of storage, the strength indicators for all groups of samples, except for those formed with the use of a 2% CH solution, significantly decreased, but did not show significant differences according to the results of statistical analysis – 21.18 ± 5.72 MPa for the CH group, 17.45 ± 3.48 MPa for the EDTA group, 18.30 ± 3.80 MPa for the DO group and 16.44 ± 2.48 MPa for the group of samples using only AS [15].

In a similar work, the effect of applying 2% CH (group I), 10% α -tocopherol (group II) and 1% quartzetin (group III) on the strength of the adhesive bond after 24 hours and 6 months of storage of samples similar to the previous study was studied using a universal AS based on the self-etching protocol. The average indicators of adhesive strength after 24 hours also did not differ significantly ($p > 0.05$) and were, on average, 13.27 ± 2.09 MPa, 14.08 ± 3.7 MPa, 13.02 ± 3.22 MPa, 15.77 ± 2.5 MPa in control, I, II and III groups, respectively. At the same time,

after 6 months of artificial aging, the same indicator in the samples made using CH was found at the level of 13.11 ± 1.92 MPa, which was significantly ($p < 0.05$) higher than the corresponding values of the control, II and III groups – 11.24 ± 2.68 MPa, 7.60 ± 4.08 MPa and 10.12 ± 3.56 MPa [16].

The results of a meta-analysis of 33 scientific works performed between 2002 and 2022 are known, which report on indicators of microtensile strength after 24 hours, 6 and 12 months of artificial aging of samples made using various MMP inhibitors. [17]. According to the authors' conclusions, only the use of 0.2% CH solution reliably increases the strength value, regardless of the generation of AS and the term of artificial aging, while the 2% CH solution did not significantly affect the adhesion parameters.

The opposite approach to creating a reliable hybrid layer is considered to be the effect on the structure of collagen dentin fibers before applying AS. A 3% solution of riboflavin, a 6.5% solution of proanthocyanidin, and a 5% solution of glutaraldehyde are used for this, which cross-link collagen fibres, increase their diameter, prevent collapse, and improve resistance to the action of enzymes, which, in turn, allows you to create a thicker and a more organized hybrid layer [3, 10, 18]. On the other hand, these substances prevent the movement of collagen molecules relative to each other under the influence of mechanical stresses arising in dentin, thus increasing the stiffness of collagen fibres [19]. A meta-analysis of 45 laboratory studies emphasized the positive effect of epigallocatechin-3-gallate, carbodiimide, EDTA, glutaraldehyde, proanthocyanidin, and riboflavin on adhesion strength after 12 months of storage, regardless of the methods of their application and pre-etching of dentin with orthophosphoric acid. At the same time, in certain works included in the meta-analysis, an immediate increase in the shear strength indicators was registered only in those samples in which the dentin surface was treated with proanthocyanidin, in contrast to the control groups of samples with traditional or modified AS application protocols [19].

In another work, on the contrary, the average strength of samples, made with a modified 3% solution of riboflavin AS, was recorded at the level of 10.16 ± 4.19 MPa after 12 months of storage, which was lower than the similar value of the control group – 13.58 ± 3.86 MPa, while the use of riboflavin in the form of a separate aqueous solution, which was applied to the surface of the dentin before the application of AS, made it possible to increase the average index of adhesive strength to 14.73 ± 6.81 MPa [10].

After treatment of the surface of demineralized dentin with a 3% carbodiimide solution, followed by

the application of V generation AS, an increase in the average shear strength was recorded from 20.89 ± 1.84 MPa in the control group of samples to 25.73 ± 1.94 MPa in the study group, and after 24 hours of storage from 18.6 ± 2.64 MPa to 24.41 ± 2.92 MPa, respectively [18].

During microtensile tests and scanning electron microscopy of samples of extracted teeth, an increase in tensile strength was determined in those samples on the dentin surface of which a 0.5% (group I) or 1% (group II) solution of phytic acid was applied for 60 seconds before the application of universal AS [20]. Average adhesive strength indicators were 38.37 ± 4.69 MPa (group I) and 35.21 ± 5.41 MPa (group II), which were significantly ($p < 0.05$) higher than the strength value of samples made without additional components of adhesive preparation – 18.80 ± 3.68 MPa. Electron microphotographs revealed the organization of a thicker hybrid layer and deeper strands of AS in the samples of groups I and II, which could be explained by deeper demineralization of dentin due to the action of phytic acid. In addition, the application of the specified solution stimulated the formation of cross-links between collagen fibers, prevented their collapse, which generally contributed to a better integration of the monomers of the universal AS to the surface of the dentin [20].

Using confocal laser microscopy, the ability of the new functional monomer N-(3,4-dihydroxyphenethyl)methacrylamide (DMA) to simultaneously form non-covalent bonds with type I dentin collagen fibres and reduce MMP activity was proven [21]. In a comprehensive study, scientists found that applying DMA for one minute to the dentin surface before applying AS increased the adhesive strength and improved the marginal fit of restorations after 10,000 thermocycles in the range from $+5^{\circ}\text{C}$ to $+55^{\circ}\text{C}$ relative to restorations made according to the traditional total etching protocol. Thus, the average tensile strength of samples with DMA was 31.85 ± 8.10 MPa, and that of samples of the control group was 22.63 ± 6.40 MPa. The value of nanoleakage of restorations, calculated as a percentage by computer analysis of microscopy data, was $21.29 \pm 4.13\%$ in the studied group, and $50.41 \pm 4.82\%$ in the control group [21]. In a meta-analysis of 7 similar laboratory studies, higher mechanical strength, lower solubility, and lower degree of water absorption of AS based on methacrylamides and urushiol were proven compared to traditional methacrylate AS immediately after the production of samples and after 6 months of artificial aging [22].

Zinc compounds also have a simultaneous effect on inhibition of MMP activity and storage of collagen fibres in dentin [23]. In addition, these compounds provide a greater degree of remineralization of dentin along the

line of the adhesive joint. In the experiment, the rate of collagen degradation was significantly lower after applying a 3% aqueous solution of zinc oxide to the surface of the dentine, and its value was almost 4 times lower than when using a 2% solution of CH. Electron micrographs of samples formed using the recommended protocol did not reveal signs of porosity and leakage of the hybrid layer [23].

According to scientists, increasing of the number of cross-links between the collagen fibres of dentin and deactivating the MMP only prolongs the service life of restorations, but does not ensure their reliability for a long time, which is explained by the gradual degradation of the hybrid layer. A more effective way to improve the quality of the adhesive bond, according to the conclusions of a meta-analysis of 8 articles, may be deproteinization and destruction of dentin collagen fibres by applying sodium hypochlorite and antioxidants after etching with orthophosphoric acid followed by the application of AS [24]. This treatment of dentin leaves a rough surface with small pits, which provides adhesion similar to that in enamel. In the works included in the meta-analysis, an increase in adhesive strength was demonstrated in samples made using a solution of sodium hypochlorite in concentrations from 5% to 10%, its exposure time from 1 to 2 minutes, V or VII generations AS [24].

A logical way to reduce the probability of the occurrence of secondary caries and, accordingly, to increase the reliability of restorations is to add agents with antimicrobial properties to AS [25-27]. In contact with the surface of such an AS, the permeability of bacterial cell membranes changes, which leads to their destruction. On the basis of a systematic review of the scientific literature on the use of antibacterial drugs in the composition of AS, the authors concluded that the highest efficiency in increasing the strength of the adhesive bond and bactericidal activity in relation to *Streptococcus mutans* is provided by universal AS modified with quaternary ammonium methacrylate [25]. In the same review, less effective antimicrobial drugs in the structure of AS are described, for example, 2% chlorhexidine, 6% sodium hypochlorite, 0.01% urushiol, dimexide, bezalkonium chloride, etc. Nevertheless, in a parallel study, no significant ($p=0.087$) difference was recorded in the strength characteristics of AS with ammonium methacrylate and conventional AS of the VII generation. After 24 hours of storage, these indicators were 17.5 ± 2.5 MPa and 12.7 ± 1.6 MPa, and after 3 months of storage in artificial saliva they were 12.3 ± 1.4 MPa and 14.7 ± 2.2 MPa, respectively [26].

The introduction of cetylpyridine hydrochloride into the AS composition allows to reduce the rate of degradation of the adhesive layer and prevent the development of caries around the restorations due to the reduction of esterase activity of oral cavity bacteria [27].

In laboratory conditions, the antibacterial effect of such AS was proven, but in a clinical study, complications in the form of secondary caries occurred even more often than with the use of traditional AS of the VII generation [3]. This can be explained by the unstable rate of release of antibacterial agents and the gradual decrease in their concentration due to chewing movements.

In the study of the growth rate of *Streptococcus mutans* biofilms on slices of samples prepared using AS doped with titanium dioxide nanoparticles, a stable and long-lasting antimicrobial effect was recorded. After 12 months of sample storage, the number of relative luminescence units (RLU) recorded by the computer analyser was more than three times lower in the samples with modified AS compared to the control group, i.e. 48,000 RLU and 164,000 RLU, respectively [1]. In another work, the values of the adhesive strength of AS with nanoparticles of titanium dioxide (group I of samples), in comparison with the same AS without additional components (group II) were studied. After 3 months, the obtained average values were 12.99 ± 2.53 MPa and 14.87 ± 2.02 MPa for the samples of groups I and II, and after 6 months of storage they were 11.37 ± 1.89 MPa and 14.19 ± 2.24 MPa, respectively [2]. Thus, the modification of AS with antimicrobial nanofillers in laboratory conditions significantly worsened the strength of the adhesive bond of the samples in the short term of the study.

Addition of penicillin V to the AS composition also enhances their antibacterial properties but does not affect the physical and mechanical properties [28]. The maximum adhesive strength for samples made with alloyed AS was, on average, $36.7\text{ MPa}\pm 10.9$ MPa, and that for samples with ordinary AS was 32.2 ± 7.9 MPa. No significant differences were found in the flexural strength indicators – 21.3 ± 1.6 MPa and 18.7 ± 3.9 MPa, the modulus of elasticity – 492.3 ± 98.4 MPa and 515.1 ± 77.7 MPa, the maximum compressive strength – 65.8 ± 11.3 MPa and 77.5 ± 7.6 MPa, respectively, for the studied and control groups of samples.

Nisin, a food additive used as a preservative, is considered another modifier proposed to increase the antimicrobial activity of AS. In the study of 4 groups of samples: the control group, where the samples were formed using V generation AS, and 3 groups in which nisin was added to AS in concentrations of 1%, 3% and 5%, it was established that the number of colony-forming units (CFU) of *Streptococcus mutans* was, on average, $0.51\times 10^7\pm 0.01$ CFU, $0.36\times 10^7\pm 0.03$ CFU, $0.34\times 10^7\pm 0.02$ CFU and $0.33\times 10^7\pm 0.04$ CFU, respectively. At the same time, the tensile strength in the same groups was 38.3 ± 2.3 MPa, 35.6 ± 2.1 MPa, 22.3 ± 1.0 MPa, and 27.1 ± 1.6 MPa [29]. Thus, increasing the concentration of nisin to 3% or 5% significantly reduces the

adhesive strength of AS, while the use of a 1% solution provides acceptable adhesive strength and pronounced antibacterial properties.

The creation of monomers resistant to all factors of destruction, i.e. hydrolytic degradation, enzymatic cleavage of organic components by esterase of bacteria, saliva, dentin, etc., is considered a promising direction of AS development [22]. At the same time, such monomers, included in the composition of AS, should not reduce their physical and mechanical properties, durability and effectiveness. Dental adhesive doped with a 5% solution of benzyldimethyldodecylammonium chloride is considered one of the examples of such modified AS. Immediately after production, the samples demonstrated a high degree of bacteriostatic and bactericidal action against *Streptococcus mutans*, maximum and specific tensile strength, hybrid layer thickness, and low cytotoxicity, similar to the classic V generation AS. However, after 10,000 thermocycles in the temperature range from +5°C to +55°C, the antimicrobial properties of such AS were lost, while the physical and mechanical properties of the samples remained unchanged [30].

CONCLUSIONS

During the restoration of defects of hard dental tissues using adhesive technologies, complications related to

the imperfection of adhesive systems often arise. To date, the development of adhesive dentistry is taking place in several directions, the most promising of which are modifications of the composition and protocols for the use of adhesive systems with the aim of deactivating matrix metalloproteinases, improving the structure of the hybrid layer due to the creation of a three-dimensional mesh of collagen fibres with optimal properties, the introduction of antimicrobial agents to slow down the growth of *Streptococcus mutans* colonies along the line of the adhesive joint.

The available results of studies of the characteristics of modified adhesive systems are often contradictory, which determines the need to develop standardized methods of their testing to obtain more reliable indicators of their physical, mechanical and biological properties. In some cases, the consequences of non-compliance with the recommendations of the manufacturing companies are a significant deterioration of the characteristics of the hybrid layer, adhesive strength, marginal fit, which, in turn, explains the need for further search for an optimized composition and techniques for applying bonding agents to improve the prognosis of restorative treatment. There are almost no results of the use of modified compositions and techniques of applying adhesive systems in clinical conditions with long periods of observation, which determines the prospects for future studies.

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CASE STUDY

ONSET OF NONSPECIFIC ULCERATIVE COLITIS POST COVID-19 (CASE STUDY)

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ABSTRACT

The patient suffered from a mild form of COVID-19 and was treated on an outpatient basis. According to the family doctor's prescription, she took Azithromycin 500 mg a day per os for 6 days, and then Ceftriaxone 1.0 g twice a day i.m. for another 6 days. Diarrhea appeared on the 10th day of treatment up to 10-15 times a day, a month later - blood admixtures in the stool appeared. The result was negative. Data from colonoscopy and histological examination of the intestinal mucosa and the clinical picture showed nonspecific ulcerative colitis, moderately severe. The patient started treatment with Salofalk first at a dose of 2 mg and then 4 mg per day. Due to the insufficient clinical effect, the patient was additionally prescribed Budenofalk in a daily dose of 9 mg with a positive clinical effect.

KEY WORDS: COVID-19, Clostridium difficile colitis, ulcerative colitis

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INTRODUCTION

On February 11, 2020, the World Health Organization gave the official name to the infection caused by the new coronavirus (SARS-CoV-2) – COVID-19 ("coronavirus disease 19"). Since then, there is daily monitoring of the epidemiology and clinical course, as well as the development and implementation of methods for the prevention and treatment of this disease.

Information on COVID-19 is constantly accumulating. SARS-CoV2 infection has been shown to mainly affect the upper airways and respiratory tract, while it often spreads beyond the respiratory system and can cause damage to other organs. In particular, a number of recent clinical reports indicate a significant damage to the gastrointestinal tract caused by SARS-CoV2 infection.

CASE REPORT

In March 2021, a 62-year-old woman sought medical advice with complaints of diarrhea 10-15 times a day with blood and mucus in more than half of the cases.

According to the patient, she got ill on December 15, 2020. Her body temperature was 38.5 °C for 2-3 days. There were no other signs of the disease. The family doctor verified the diagnosis of COVID 19 by detecting the genome of SARS-CoV-2 virus in nasopharyngeal lavage by reverse transcription polymerase chain reaction (RT-PCR), performed using the HEMA test system on

KING FISHER DO analyzer with a sensitivity of 5 copies per reaction. Wheezing was not heard in the lungs. Saturation according to the outpatient examination was 99%. The results of thoracic computed tomography are shown in Fig 1. The following antibiotics were prescribed: Azithromycin 500 mg per os for 6 days, then – the same duration of treatment with Ceftriaxone 1.0 g i.m. twice a day. After 10-12 days from the onset of the disease, diarrhea mixed with blood appeared 10-15 times a day. The patient was referred by a family doctor to a gastroenterologist, where she was prescribed Salofalk in granules 1 sachet once a day, Linex, Normagut 1 capsule twice, Creon 25,000 IU three times a day. The patient's condition did not improve: bloody diarrhea continued up to 10 times a day.

We know from the patient's life history that in 1979 she suffered from pulmonary tuberculosis, was treated in a dispensary and in follow-up for 10 years. No allergic reactions to medication mentioned. Concomitant diseases denied.

On examination: the patient is with a regular build, well-nourished. Weight – 64.0 kg. Height – 162 cm. BMI 24.4 kg/m². The skin is clean, pale pink, visible mucous membranes are clean. Peripheral edema is absent. Musculoskeletal and muscular system is normal. Upper and lower extremities are within normal limits. Head, eyes, ears, nose, oropharynx, neck, nervous system, lymph nodes without objective signs of pathology. Nervous

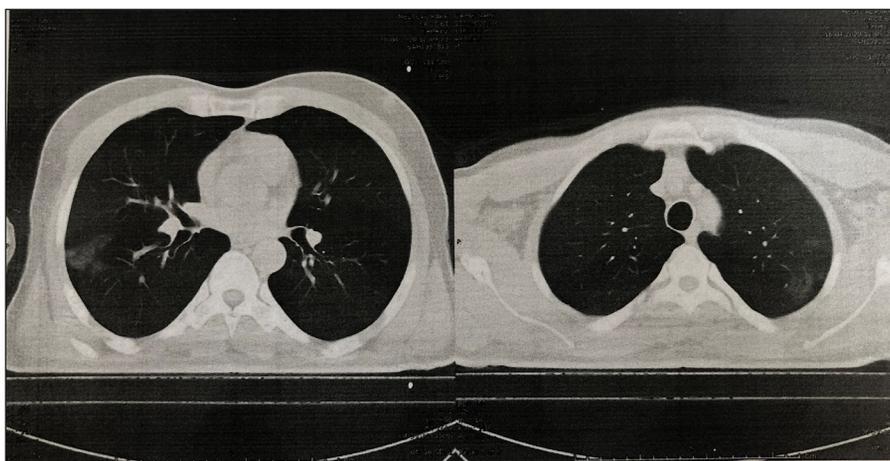


Fig. 1. Computed tomography of the lungs with COVID-19



Fig. 2. Colonoscopy from 17.02.2020. Signs of sigmoiditis

system is within normal limits. Evaluation of physical data was performed in a sitting position. Body T (axillary) is 36.5 °C. The thyroid gland is not enlarged. Breathing is clean, vesicular in the lungs. Respiratory rate – 17. Heart tones are rhythmic, clean, loud. Blood pressure and heart rate in a sitting position were 127/84 mm Hg. and 78 per minute respectively. Abdomen is of normal shape, freely involved in the act of breathing, soft, painful on palpation in the left iliac region. Liver is at the level of the costal arch. The kidneys are not palpable, Pasternak's symptom is negative, urination is not disturbed.

The laboratory and instrumental methods of examination were performed. Blood tests: HB 140 g/l, erythrocytes $4.7 \times 10^{12}/l$, leukocytes $4.9 \times 10^9/l$, platelets $246 \times 10^9/l$, Ht 0,41 V/V, blood formula: neutrophils abs $2,48 \times 10^9/l$, lymphocytes $1.62 \times 10^9/l$, monocytes $0.59 \times 10^9/l$, eosinophils $0.20 \times 10^9/l$, basophils $0.01 \times 10^9/l$. Biochemical parameters: glucose 4.8 mmol/L, protein total 70 g/L, albumin 47 g/L, bilirubin total 7 μmol/L, ALT 22 U/l, AST 25 U/l, creatinine 64 μmol/L, hsCRP 1.56 mg/L. PT 11.0s, INR 0.95, aPTT 31.0s.

Colonoscopy from 17.02.2020: examination was performed to the head of blind colon with intubation of

the terminal part of the small intestine. There is a small amount of light content in the lumen. A formation with a diameter of up to 3 mm was verified in the head of blind colon; a polypectomy was performed by the cold method and a biopsy slice was taken for histological examination (No.1). Changes of the mucous membrane 10 cm long with submucosal hemorrhages and small erosions were detected in the sigmoid colon at a distance of 40 cm from the anus. Biopsy slices (No.2) were taken from this zone. Erosions with submucosal hemorrhages (No.3) were also detected in the rectum. No pathology detected in the rectal ampulla. Conclusion: polyp of the head of blind colon. Sigmoiditis.

Histological examination of biopsy slices:

No.1. A fragment of the mucous membrane of the colon with a fragment of the proper muscle plate. The superficial epithelium is focally desquamated. The number of crypts in the own plate of the mucous membrane is reduced. In part of the crypts, there are crypts and crypt abscesses with focal destruction of crypts, atrophy of goblet cells. Singular lymphoid clusters with germinal centers are present in the proper muscle plate; diffuse lymphocyte-macrophage

infiltrate with granulocyte impurities around. Proper muscle plate is not thickened.

No. 2. Fragments of the mucous membrane. Crypts are unevenly distributed in the proper plate. Focal surface erosions, crypts and crypt abscesses. The own plate of a mucous membrane contains a diffuse lymphocyte-macrophage infiltrate with admixtures of leukocytes.

No. 3. Fragments of the mucous membrane. Crypts are unevenly located in the proper mucous plate. Focal crypts, crypt abscesses. Focal erosions of superficial epitheliocytes. Focal fibrosis in the proper mucous membrane.

Conclusion: changes in the mucous membrane of the colon, characteristic of nonspecific ulcerative colitis.

In order to rule out intestinal infection in the patient, in particular clostridial infection, a fecal examination was performed for STOOL CULTURE. Results: Salmonella No Salmonella Isolated, Shigella No Shigella Isolated, Yersinia No Yersinia Isolated, Campylobacter No Campylobacter Isolated, E. coli 0157 No E. coli 0157 Isolated. C. difficile Antigen/Toxin Negative for GDH Antigen and Toxin A/B, Ova & Parasite – No pathogenic Ova or Parasites detected. The results were negative. Calprotectin fecal was significantly elevated in the patient – 4738.29 mg/kg.

Therefore, the patient was diagnosed with: convalescence after Coronavirus disease, nonspecific ulcerative colitis, high activity, moderate severity (9 points on the Mayo Score).

Due to the ineffectiveness of previous treatment (only 1 g of Salofalk), the dose was doubled and Budenofalk was additionally prescribed at a dose of 9 mg per day. After 2 weeks, the patient's condition improved: the number of bowel movements decreased to 3-4, but blood admixtures in the stool remained. The patient continues to this treatment. Monitoring of the patient has been extended. During the second visit 2 weeks after, there was no blood in the stool and the number of bowel movements decreased to 2 per day.

The peculiarity of this case is the onset of nonspecific ulcerative colitis, provoked, possibly, by Coronavirus disease and/or unjustified long-term antibacterial therapy. Reports on the development of nonspecific ulcerative

colitis appeared in the first months of the Coronavirus pandemic [1]. The causative agent of Coronavirus disease SARS-CoV-2 has long been detected in biopsies of the stomach, duodenum, rectum and feces, even when it is no longer found in sputum, which proves the persistence of the virus in the digestive tract [2]. Recent publications suggest that the COVID-19 pandemic is a stressor that may be a provocative factor for the manifestation of latent ulcerative colitis [3]. Such patients should continue to be treated as they are treated without Coronavirus disease [4]. In both cases, according to an authoritative international document, treatment with 5-ASA medications should be continued. However, there are caveats that apply to patients taking systemic corticosteroids (more than 20 mg per day) who need to reduce their dose as soon as possible (10 mg weekly), and to patients on combination therapy (immunosuppressive with biopharmaceuticals), in which the dose of immunosuppressive drugs should be reduced. However, it is not yet clear whether treatment with Budesonide, anti-tumor necrotic drugs, Vedolizumab, or Ustekinumab should be stopped in asymptomatic patients. On the other hand, treatment with thiopurines (Azathioprine, 6-Mercaptopurine), Methotrexate, and Tofacitinib should be stopped in patients infected with SARS-CoV-2, although symptoms of NUC remain [4]. According to other recommendations, after two negative PCR tests, patients with NUC can continue its treatment [5].

CONCLUSIONS

Thus, we consider 2 options for a combination of nonspecific ulcerative colitis and Coronavirus infection: 1) when a patient with asymptomatic NUC has SARS-CoV-2 infection, which leads to the manifestation of inflammatory bowel disease; 2) when the Coronavirus disease causes the development of NUC. The shortcoming of our study was that we did not perform genetic testing in the diagnosis of ulcerative colitis in order to reveal which version of the development of events our patient had.

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CASE STUDY

EXTRASKELETAL EWING'S SARCOMA IN PEDIATRIC PATIENT RARE CASE: A CASE REPORT

DOI: 10.36740/WLek202312126

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ABSTRACT

This study reports a 12-year-old male was referred to our institute with a mass in the right ear. The mass was rapidly growing thus needing an immediate surgical attention. MRI revealed an exophytic, hyper vascular mass extending to the right jugular fossa and, the middle ear, and to the right acoustic meatus, suggestive of a right glomus jugulare tumor. Angiography showed a hypervascular lesion with robust feeding from the superficial temporal artery and right occipital artery. After surgery, the patient rapidly recover and he was discharged home on the 5th day after surgery. Three months after surgery, the lump reappeared and grew rapidly as before.

KEY WORDS: Extraskelatal sarcoma, Ewing sarcoma, sarcoma, Intracranial invasion

Wiad Lek. 2023;76(12):2733-2737

INTRODUCTION

Ewing sarcoma/peripheral primitive neuroectodermal tumor (ES/pPNET) is an undifferentiated malignant, small, round cell tumor which rarely occurs in the skull and meningeal tissue[1]. It accounts for 4-10% of all primary bone cancers affecting teenagers and young adults and rarely develops after age 30 years [2]. According to anatomical site of occurrence it is classified as : (a) intraosseous (most common) (b) extraskelatal (less common) and (c) periosteal (rare) type. Intracranial invasion of extraskelatal ES extremely rare [3].

Intracranial invasion of extraskelatal ES is often misdiagnosed as an atypical teratoid/rhabdoid tumor (AT/RT) or a primary leptomenigeal medulloblastoma [2]. In addition, because of the lack of clinical symptoms in the early stages of cranial ES, most patients are diagnosed in advanced stages, leading to worse outcomes [4]. In this case report we present a case of a 12-year-old male with intracranial invasion of extraskelatal ES.

CASE REPORT

A 12-year-old male was referred to our institute with a mass in the right ear for 5 months prior to admission. The progression of the mass size was rapid. He also had persistent headache and ear pain. Clinical examination revealed paresis of the 7th cranial nerve (House-Brackmann 2), bilateral papilledema and conductive hearing

loss of the right ear. Motor strength, sensory and reflexes were normal.

Radiological workup with MRI revealed an exophytic, hyper vascular mass extending to the right jugular fossa and, the middle ear, and to the right acoustic meatus, suggestive of a right glomus jugulare tumor. The mass was supplied by a branch of external carotid artery.

Angiography (Fig 3.) showed a hypervascular lesion with robust feeding from the superficial temporal artery and right occipital artery. Laboratory examination was conducted, there is no abnormalities in the pre-operative lab result. Excision of the tumor was conducted, and the patient's recovery went well.

OPERATIVE MANAGEMENT

Intraoperatively, the tumor was found to have invaded the dura and the cranium. The involved bone (including the lateral sphenoid wing) and the large dural cuff were resected. The tumor was soft and hypervascular.

The patient's postoperative recovery went well and he was discharged home on the 5th day after surgery. Three months after surgery, the lump reappeared and grew rapidly.

HISTOPATHOLOGICAL EXAMINATION

Histopathological examination showed round to oval cells arranged in lobules, separated by a thin vascular



Fig. 1. The appearance of the mass at the first visit in the ENT outpatient clinic (a), 1 month after the first visit, the lump was increasingly visible from outside of the ear canal (b), two months after the first visit the lump was visibly protruding from the ear canal with necrotic parts (c)

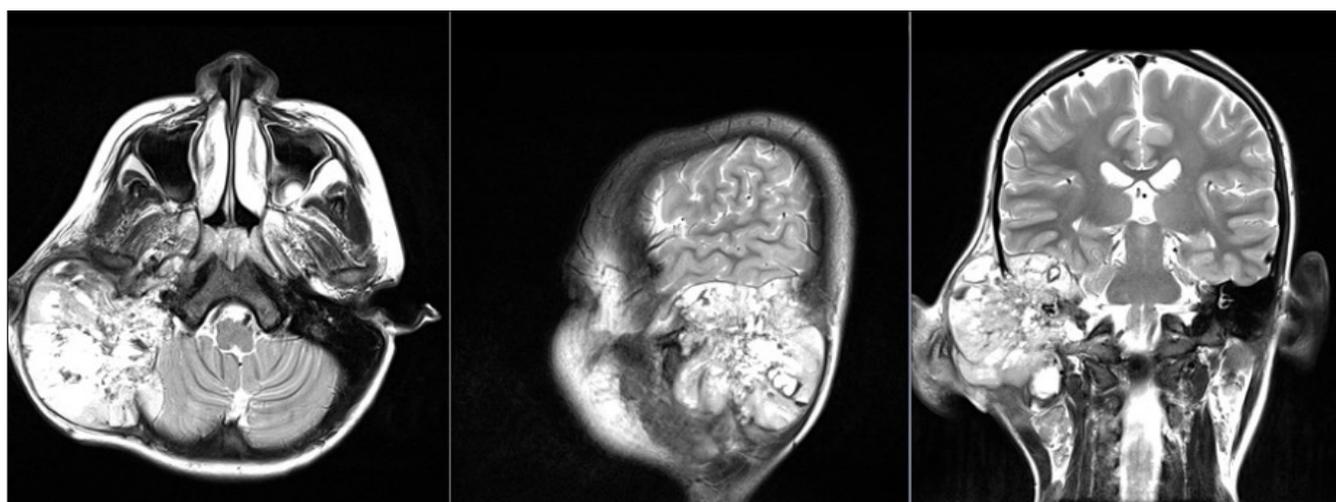


Fig. 2. MRI of the patient. T2W1 with contrast.

channel, with vesicular nuclei. Mitotic figures were observed with foci of necrosis. The immunohistochemistry positive for CD99 but negative for CD20, CD3, myosin and glial fibrin acid proteins. The overall features are consistent with a primary ectodermal neuroendocrine tumor (PNET), suggesting an Ewing sarcoma in the temporal region.

ADJUVANT TREATMENT

After the result of pathological anatomy with IHC CD99 was reported, chemotherapy immediately conducted. The chemotherapy regimen consisted of vincristine-doxorubicin-cyclophosphamide. However, before the chemotherapy regimen is finished, the clinical condition of the patient is deteriorating. The patient

unfortunately passed away before receiving any radiotherapy treatment.

Cases of the central nervous system extraosseous Ewing's sarcoma (ES) are extremely rare. Only few that have been reported literature [5]. Jay might be the first to describe a patient with an isolated posterior fossa mass that histologically resembles a medulloblastoma [6].

Paulus mentioned in their study in 1991 that out of 2500 cases of brain tumor, only 9 were sarcomas, among which only one was reported as ES [7]. Similarly, Krishnamani et al. reported only 7 cases were primary ES of the skull out of 332 cases of ES of any sites [8]. These tumors clinically present with signs of increased intracranial pressure. Motor deficit or visual disturbances were uncommon and endocrine abnormalities can occur depending on the tumor location.

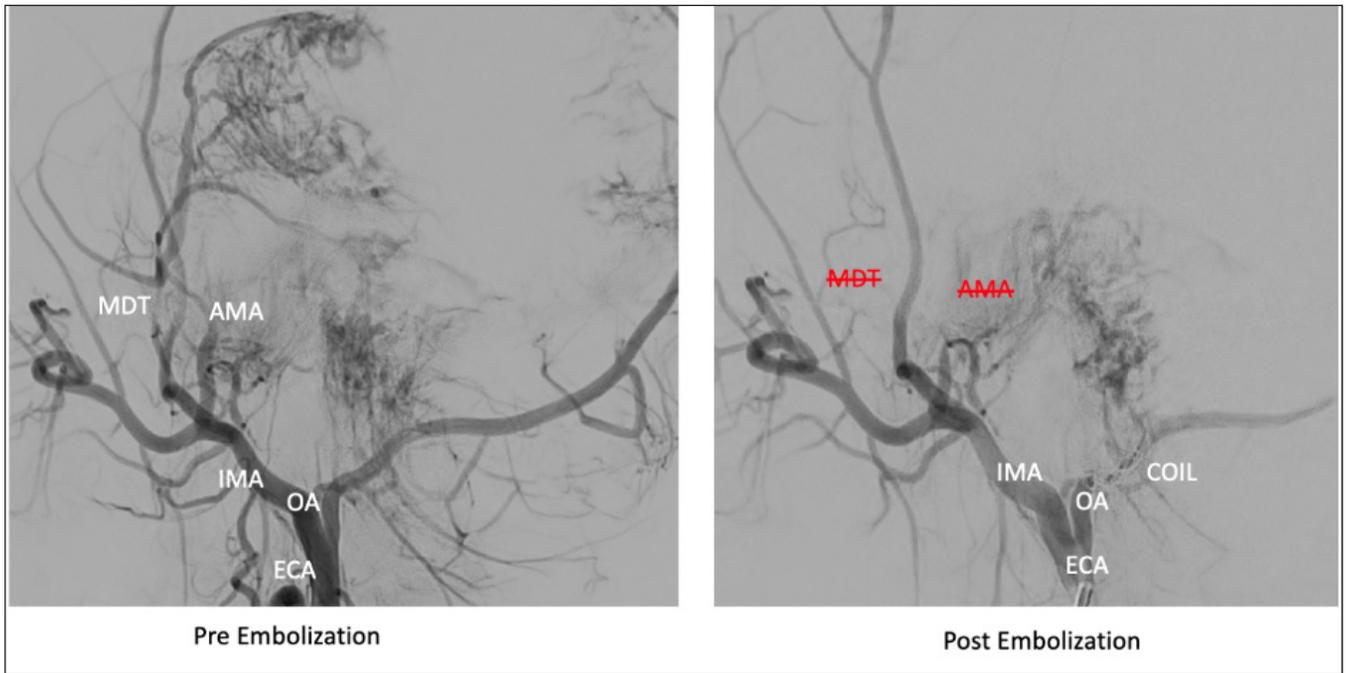


Fig. 3. Angiography study of pre-embolization and post embolization.

MDT: Middle deep temporal; AMA: Accessory Meningeal Artery; IMA: Inferior Meningeal Artery; OA: Occipital Artery; ECA: External Carotid Artery.

Diagnosis of ES requires a histopathological examination, immunohistochemistry, and cytogenetics. The differential diagnosis of this intracranial round cell tumors is primitive neuroectodermal tumor (neuroblastoma), lymphoma, rhabdomyosarcoma, and Ewing's sarcoma. Macroscopically, intracranial invasion of extraskeletal ES might mimic meningioma due to its dural base and bone-eroding manner, as presented in our case.

Radiographs may show a nonspecific soft tissue mass. Computed tomography (CT) usually shows a soft tissue mass with attenuation similar to that of skeletal muscle [9]. In our patients, head CT Scan showed a rounded, poorly-defined, heterogeneously enhancing lesion in the right temporal region, with a mass effect and destruction of the right temporal bone, extending into the scalp. Head MRI revealed a solid mass from right jugular fossa to right external acoustic meatus, which appears isointense on T1-weighted images, hyperintense with prominent flow voids on T2-weighted-images. In patient with ES, MRI imaging usually revealed a mass with signal intensity similar to that of skeletal muscle on T1-weighted imaging. On T2-weighted images, the mass often demonstrates a heterogeneous intermediate to hyperintense signal. Hyperintense areas representing foci of cystic or necrotic changes can be found. High-flow vascular channels or flow voids may also be seen which is extremely common, although not unique to ES [9].

A report by Deshpande et al., described 8 similar case with the age range of 1 to 33 years, with the median

age of 9 years. Clinical manifestation in this report also varied greatly ranging from seizure, ptosis, weakness in extremity, but headache, and vomiting were the most reported complaint [10]. This is similar to our case which described a 12 year old male with solid mass at right jugular fossa which also complained of headache and showed facial palsy as neurological deficit.

Huang et al., reported a case that is similar to our case. In this report, a 19 year old patient is admitted with a complaint of headache, vomiting, and behavioural changes. Neurological examination is remarkable with an exception in right pronator drift. MRI showed a large extra-axial mass which is heterogeneously enhanced on the left frontal convexity [10].

Another report by Jiang et al, described a 55 year old female with a chief complaint of memory decline over 1 month with hemiparesis. In this case, MRI revealed a large irregular mass located in the left frontal lobe with mixed isointense-to-hypointense signals on T1-weighted imaging, heterogenous hypointense-to-hyperintense signals on T2-weighted imaging, and T2 dark-fluid [7].

The exophytic part of the tumor protruding to the outside is the most interesting point in our finding case. The presentation extra skeletal ES which protruding from intracranially to the outside to the ear canal has not been reported. It differs in presentation from other previously reported case by Huang et al., that revealed as a well-defined mass with dural or bony involvement, mimicking the appearance of a meningioma [11]. An-

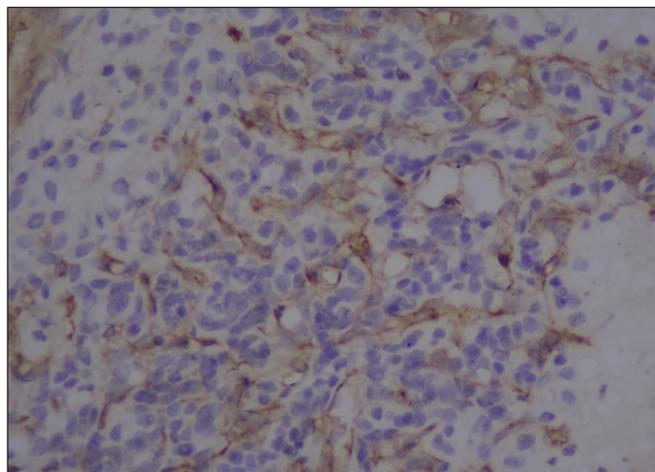


Fig. 4. EWS / PNET's are characterized Immunohistochemistry revealing strong positivity of tumour cells to the surface antigen CD99.

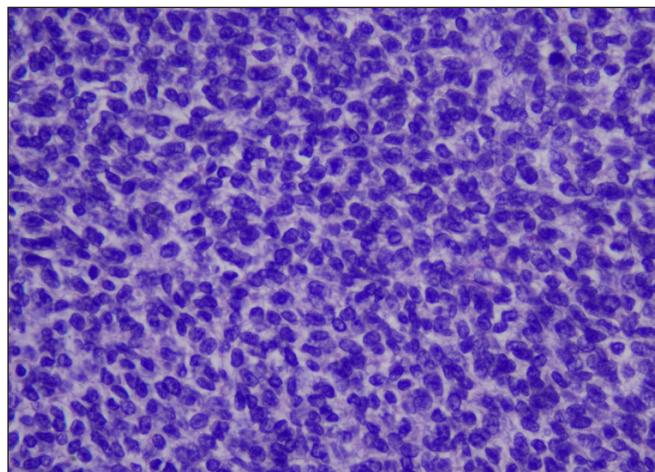


Fig. 5. Hematoxylin and eosin stain of typical EWS/PNET's at 20× magnification. Notes: Uniform small cells with round nuclei and fine chromatin are seen.

other study by Alan et al., reported the radiographic finding of their patient, demonstrating a circumscribed homogenous subcutaneous mass with no erosion of the calvarium [9]. The presentation of cranial ES is heterogeneous, and awareness of extra skeletal ES as one of the differential diagnosis may aid in better management and outcome for patient.

This case is extremely rare, describing a patient with a rapidly progressive intracranial invasion of extraskelatal ES. Diagnosis of glomus jugulare was assumed, and excision of tumor was conducted with excellent early post-operative condition. However, a rapid progression of regrowth should alert a clinician that a malignant mass might be the cause of this condition. Pathological examination revealed ES, an aggressive tumor of

bones and soft tissue. Radiotherapy was then planned for this patient. Unfortunately, since the waiting list for radiotherapy in our institution is quite long, the patient passed away before receiving further treatment. An early diagnosis and management in the suspicion of central nervous system ES should be done.

CONCLUSIONS

Extraskelatal ES is rare tumor with nonspecific clinical presentation and radiological features. It is locally aggressive and requires multimodality treatment with surgery and adjuvant chemoradiation therapy. Early diagnosis and treatment of intracranial invasion of extraskelatal ES may aid in better management for patient.

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CASE STUDY

SARS-COV-2 INFECTION AS A POSSIBLE TRIGGER FOR MICROSCOPIC POLYANGIITIS: CASE REPORT AND MINI-REVIEW

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ABSTRACT

The paper presents a clinical case of MPA in a 67-year-old woman following COVID-19, characterized by significant difficulties when working with the early etiological verification of diagnosis. The patient presented with polyarthritis affecting the upper and lower limbs, fever, and comorbid urological pathology in the form of urolithiasis and recurrent cystitis. This clinical presentation, hyperuricemia, azotaemia and anemia were mistakenly interpreted as chronic kidney disease: gouty nephropathy, gouty arthritis, which masked the underlying disease for a long time delaying the timely MPA diagnosis and treatment. Given that MPA is a multisystemic disease, it is essential to enhance awareness and knowledge of healthcare professionals of various specialties regarding AAVs and MPA in particular, as evidenced by the online survey data during COVID-19 pandemic among doctors in 21 countries.

KEY WORDS: ANCA-associated vasculitis, microscopic polyangiitis, clinical presentation, COVID-19, novel SARS-CoV-2 infection

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INTRODUCTION

Microscopic polyangiitis (MPA) is a necrotising vasculitis of small vessels, one of the clinical phenotypes of a group of rare systemic diseases known as vasculitis associated with antineutrophil cytoplasmic antibodies (ANCA). ANCA-associated vasculitis (AAVs) encompass a heterogeneous group of disorders characterized by severe destructive systemic vasculitis of small vessels and the development of autoantibodies against neutrophilic proteins, especially myeloperoxidase (MPO-ANCA) or proteinase 3 (PR3-ANCA). Currently available tests for MPO-ANCA and PR3-ANCA are highly sensitive and specific serological markers for the diagnosis of MPA and granulomatosis with polyangiitis (GPA) [1].

The main clinical and pathological variants of AAVs are MPA, GPA (formerly known as Wegener's granulomatosis), eosinophilic granulomatosis with polyangiitis (formerly Churg-Strauss syndrome) and single-organ AAV (such as renal-limited AAV) [2].

MPA was officially recognised at the first International Consensus Conference Chapel Hill Consensus Conference in 1994, which introduced the term MPA, referring to pauci-immune (i.e. minimal or absent immune deposits) necrotising vasculitis affecting small vessels with or without involvement of medium-sized arteries [3].

Despite ANCA-associated vasculitis remaining a rare autoimmune disease, the incidence and prevalence of

AAV overall, including MPA have increased over the past 30 years [4, 5]. According to updated epidemiological data, the average standardized incidence rate for MPA per million was 5.04 in the 1990s, increased to 9.2 in the 2000s [5] and continues to rise. Our own clinical experience confirms this increase both before and after the COVID-19 pandemic [6].

The global COVID-19 pandemic caused by the novel coronavirus infection SARS-CoV-2 has been a major concern for the global medical community in recent years. It is known that apart from respiratory syndrome, the SARS-CoV-2 virus causes damage to the cardiovascular system, kidneys, joints, nervous system, etc., significantly affecting the clinical course of the disease and mortality. Furthermore, studies by Varga et al. indicate the presence of direct viral infection of endothelial cells of numerous organs and diffuse endotheliitis in COVID-19 patients leading to widespread endothelial dysfunction associated with apoptosis [7]. Cytokine-induced endothelial inflammation and vascular pathology in COVID-19 have been well described in post-mortem biopsies and several clinical cases reporting micro/macro thrombotic events in small, medium, large vessels and vasculitis in multiple organs [8-10].

Furthermore, there is growing evidence that SARS-CoV-2 is another virus which can trigger the emergence of new or exacerbate existing autoimmune diseases in children and adults [10-14].

Recently, the scientific literature has been increasingly reporting on a potential link between SARS-CoV-2 infection and AAVs: firstly, because lung lesions in COVID-19 can mimic the changes observed in patients with AAVs [14-17]; secondly, two diseases can occur concurrently; and thirdly, COVID-19 can trigger AAV [14-17]. The development of AAV following COVID-19 has been reported in many clinical case reports [10, 14, 16-19]. It is quite obvious, that diagnosing new cases of AAVs in a patient with COVID-19 is a challenging task for physicians, since some symptoms and clinical manifestations are common to both conditions [14].

Most published reports have focused on clinical case reports regarding the occurrence of two AAVs serological types associated with MPO-ANCA or PR3-ANCA without specifying the clinical phenotype of ANCA-associated vasculitis in patients who have experienced a novel coronavirus disease. Moreover, it should be emphasised that only a few publications specifically address new cases of MPA development following SARS-CoV-2 infection [8, 20-22].

Thus, the rarity of AAVs in general and MPA in particular, poorly known to a wide range of practitioners, the increasing incidence in recent years, diverse clinical manifestations of the disease and lack of specific symptoms, similarity to other syndrome-like diseases, the complexity of early diagnosis and management of such patients contribute to the practical relevance of this problem in everyday medical practice. The problem of AAVs and MPAs is especially relevant in the period of the novel SARS-CoV-2 coronavirus infection, given the common anatomical areas of COVID-19 and systemic inflammation [23].

CASE REPORT

A 67-year-old patient A. was admitted to the Nephrology and Dialysis Centre of Poltava Regional Clinical Hospital (PRCH) on April 22, 2022 with complaints of general weakness, intermittent heaviness in the lumbar region, pain in the knee, hip, elbow, shoulder and wrist joints, as well as feet. She felt ill for about two months, when after COVID-19 (verified by PCR) in February, she developed pain in the lower back and above-mentioned joints, their swelling, pain in the chest and along the spine, an increase in body temperature to 37.4-37.8 °C, and general weakness. The patient self-administered NSAIDs which provided temporary relief.

At the end of February her family doctor prescribed other NSAIDs which did not improve her condition. In April, the joint pain intensified, and she was treated by rheumatologist, who suspected rheumatoid arthritis. After additional examination (X-ray of the hands and

feet, biochemical blood tests for inflammatory markers, rheumatoid factor, anti-cyclic citrullinated peptide antibodies), the diagnosis of rheumatoid arthritis was excluded. The diagnosis was secondary arthropathy involving the shoulder, elbow, hip, knee, ankle joints and II functional disability level. When examining, additional findings detected azotaemia (creatinine 300 µmol/l), anemia, hyperuricemia (HUA), which led to the referral of the patient to a nephrologist at PRCH and hospitalization to the Nephrology and Dialysis Centre.

Medical history data: patient experiences recurrent cystitis, urolithiasis (UL) for over 3 years. She received urological outpatient treatment, but did not undergo biochemical blood test. She has been diagnosed with autoimmune thyroiditis and hypothyroidism for the past 8 years and takes replacement therapy. Physical examination revealed tenderness on percussion over the sternum and ribs as well as when palpating the shoulder, elbow, hip, knee joints, and at the areas of the rib attachments to the sternum; ankle joints were painful on palpation, swelling of the ankle and foot joints; the skin over them was unchanged, without local temperature increase. Passive and active joint movements were limited. Bp – 160/90 mmHg, pulse rate – 80 bpm., rhythmical and satisfactory.

The patient was tested for chronic hepatitis markers (HBV, HCV), HIV infection – the results were negative. PCR testing for SARS-CoV-2, taken from the nasopharynx and oropharynx was negative. Changes in laboratory parameters during hospitalization detected: complete blood count (CBC) – anemia (Hb – 89-86 g/l, erythrocytes – 2.95-2.98·10¹² /l) lymphopenia (12%), a sharp increase in ESR (61-62 mm/h); BAC – signs of azotaemia (creatinine 360.2-293.8 µmol/l, urea 21.8-26.4 mmol/l); HUA (uric acid 557.2-395.5 µmol/l), hypercholesterolaemia (total cholesterol (TC) 5.26-6.3 mmol/l), hypocalcaemia (Ca 1.23-1.24 mmol/l), hyperfibrinogenemia (5.2 g/l), elevated ferritin levels (208.4 ng/ml with a normal range of 12-150 ng/ml for women), decreased serum transferrin level – 1.51 g/l (normal range: 1.7-4.7 mg/l).

General urinalysis (GUA) detected the signs of urinary syndrome: proteinuria (0.885-1.88 g/l), erythrocytes – unchanged from to 40 per ppm, leukocytes – 8-14 per ppm. The Zimnitskyi test showed a specific gravity 1007-1014, nocturia (daytime diuresis 620.0 ml, and night-time diuresis 1170.0 ml). Daily proteinuria: 1.39 g/day. ECG revealed sinus rhythm and leftward deviation of EKV, signs of left ventricular hypertrophy.

Ultrasonography of the abdominal cavity and pancreas detected ultrasound signs: GI, chronic cholecystitis, pancreatitis, nephritis, urolithiasis of both kidneys, cysts of both kidneys. X-ray of hands and

feet indicated Rø signs of stage II-III polyosteoarthritic changes, particularly affecting the metatarsophalangeal joint of the first toe on the left foot). FGDS revealed duodenal-gastric reflux. The patient was examined by related specialists, including rheumatologist, haematologist, urologist and their conclusions were included in the clinical diagnosis. The patient refused from sternal puncture. The clinical diagnosis determined: CKD IV (GFR by SKD-EPI – 15 ml/min/1.73m²): secondary gouty nephropathy in combination with pyelonephritis, latent course; UTI: small calculi in both kidneys; arterial hypertension, grade 3, very high risk; moderate severity anemia of chronic patient; chronic gouty arthritis; duodenal gastric reflux; chronic calculous cholecystitis; chronic pancreatitis, latent course. The patient was prescribed detoxification, antihypertensive, antihypertensive, membrane-stabilising, anticoagulant, antioxidant, antineoplastic therapy, as well as anti-inflammatory and anti-anemic drugs. In early May, the patient was discharged with slight improvement: the lumbar pain subsided and the joint pain decreased.

In the following period, the patient was regularly monitored by rheumatologist, nephrologist, urologist, gastroenterologist and sought medical care from other related specialists. In particular, in May, she visited neurologist regarding myofascial pain syndrome and dorsalgia. In May-June, she received treatment from dermatologist for eczema-like rash on her right hand and foot. The diagnosis was pustular psoriasis. At the end of June, the patient was consulted by a cardiologist for shortness of breath and chest pain. The diagnosis was made based on the examination data, namely, metabolic cardiomyopathy, stage II arterial hypertension, grade 3, high risk; atrial fibrillation. After treatment, the patient's condition improved somewhat, but she continued to experience joint and rib pain and pain along the spine. Taking into account her complaints, chest X-ray was performed, which revealed radiological signs of bilateral (more left-sided) hydrothorax, left-sided upper lobe pneumonia. She was hospitalized and treated in the therapeutic department.

In September, the patient visited neurologist again regarding the chest pain and appearance of shooting pain in the spine. She was diagnosed with chronic thoracalgia, disseminated encephalopathy with bilateral reflex insufficiency. MRI of the spine was recommended, which indicated signs of left-sided C-shaped scoliosis of the thoracic spine, grade 1; osteochondrosis of the thoracic spine; B Th4 haemangioma; intracorporeal Schmorl's nodes; deforming spondylosis, spondylarthrosis of the thoracic spine; bilateral hydrothorax.

In October 2022, the patient noticed a deterioration in her health, namely, increased pain in the joints and

their swelling, frequent night-time urination (up to 3 times), which led her to visit a nephrologist. Blood tests showed creatinine of 350 µmol/l, urea – 15.4 mmol/l, which made it possible to diagnose gouty nephropathy. The patient was hospitalized at the regional nephrology centre for further examination and treatment.

At the time of admission on November 8, 2022, the patient complained of pain in the shoulder, elbow, hip, knee, ankle joints, swelling in the feet and lower legs, acute pain along the spine, general stiffness of movements due to pain, inability to take deep breaths, shortness of breath with minimal physical exertion (such as dressing and getting up), periodic squeezing pain in the heart area, increase in the body temperature up to 37.4 °C, weight loss of 12 kg over 4 months, general weakness, occasional dizziness. Objectively: shoulder, elbow, hip, knee, ankle joints were painful on palpation, swelling of the ankle and foot joints was observed. The skin over the joints showed no changes, without local temperature elevation. Passive and active joint movements were moderately limited. Patient experienced pain in the bones of the hands, legs and ribs on palpation.

The data of inpatient examination revealed anemia (Hb – 102-98 g/l), increased ESR (54-47 mm/h), azotaemia (creatinine 328.7-303.7 µmol/l, urea 20.7-26.9 mmol/l), HUA (uric acid 524.5 µmol/l, despite taking the drug febuxostat), hypercholesterolaemia (FPG 6.4 mmol/l), hypocalcaemia (Ca 1.21 mmol/l). A sternal puncture was performed without deviation from the normal range.

The urinalysis detected moderate proteinuria (0.488-0.657 g/l), microhaematuria (unchanged erythrocytes from 9-11 to 20-25 per day), mild leukocyturia (1-3 to 10-12 per day). Daily proteinuria was 1.09 g/day. Urine culture for microflora showed no growth of aerobic bacteria.

ECG: sinus rhythm, heart rate 69 beats/min. ECV deviated to the left, incomplete blockade of the anterior left branch of the His bundle, signs of LV strain, paired supraventricular extrasystole. Echocardiography detected adequate contractile function of LV. The heart cavities were not enlarged. Initial hypertrophy of the LV myocardium with type I diastolic dysfunction, changes in the aorta and aortic valve with regurgitation of I degree, regurgitation of the MC, TC of the I degree were noted. LVEF was 62 %. CT scan of the thoracic cavity organs showed signs of left-sided hydrothorax, hydropericardium, left-sided upper lobe pneumonia. FGDS revealed erythematous gastroduodenopathy, duodenal-gastric reflux.

Immunological blood tests dated November 16, 2022 detected myeloperoxidase (MPO), Ig G antibodies

(ELISA) – 192.36 U/ml (normal < 20 U/ml); antinuclear antibodies (ANA, IFT method) – 1:1000 (normal < 1:100); double-stranded DNA (ds DNA), Ig G antibodies – 1.5 IU/ml (normal < 10.0 IU/ml, negative result).

Thus, the test data can be interpreted as verified systemic ANCA (MPA+) vasculitis. The patient was examined by related specialists: rheumatologist, haematologist, gastroenterologist, pulmonologist, endocrinologist, urologist, neurologist, psychiatrist, angiologist. It is worth mentioning that after discharge from the hospital in early May, the patient visited various related specialists a total of 20 times, and was consulted by a psychiatrist who ruled out psychiatric pathology. The psychiatrist's conclusion: astheno-neurotic syndrome.

It should be noted that the diagnosis of MPA was substantiated in accordance with the new criteria MPA classification ACR/EULAR 2022 [24], which are now confirmed for the use in clinical trials. These criteria assign the highest score to MPO-ANCA (or perinuclear ANCA, pANCA).

DISCUSSION

Systemic vasculitis, ANCA associated: microscopic polyangiitis (MPO+), grade III activity, chronic course, with kidney damage (CKD V (eGFR SKD-EPI=12 ml/min/1.73m²): rapidly progressive glomerulonephritis, urinary syndrome; arterial hypertension, grade III, very high risk); pulmonary involvement (hydrothorax, pneumonia, pulmonary fibrosis, grade II); cardiac involvement (pericarditis, myocarditis, CHF IIA, FC III); central nervous system involvement (dyscirculatory encephalopathy with bilateral reflex pyramidal failure); peripheral nervous system (polyneuropathy); joints (polyarthralgia). Associated pathologies corresponded to the previous clinical diagnosis. Prescribed pathogenetic treatment included pulse therapy: "Solu-Medrol" (methylprednisolone) 250 mg IV drip No. 3, "Endoxan" (cyclophosphamide) 400 mg IV drip 1 time in 2 weeks (in the morning) in 200 ml of 0.9% sodium chloride solution, and then 1 time in 3 weeks 3-4 months (depending on the patient's condition), mesna solution (for prevention of toxic effects of cyclophosphamide on the urinary tract): during endoxan infusion, 4 hours and 8 hours after the infusion; after "Solu-Medrol", switch to "Medrol" 32 mg tablets per day with further dose reduction according to the regimen. Additionally, the patient received detoxification, antihypertensive, antianemic therapy, as well as hepatoprotectors, sorbents.

She was discharged from the hospital with significant improvement (in the patient's words, "born again") to continue outpatient treatment under the supervision of a multidisciplinary team of specialists (family doc-

tor, nephrologist, rheumatologist, etc.). Currently, she continues to receive pathogenetic therapy and feels satisfactory.

It is widely acknowledged that the novel coronavirus disease 2019 (COVID-19) has become a global problem since its outbreak in December 2019, as it is associated with a great number of pathologies, affecting numerous organs. Moreover, SARS-CoV-2 is associated with the development of rheumatic diseases, especially small vessel vasculitis and arthritis. Typically, the onset occurs in several days or weeks after antigenic infection and in patients with mild COVID-19 [10, 11]. Based on the data of the analysed literary sources in the PubMed database, we determined that in 40% of cases AAV was diagnosed 1-6 months after COVID-19, while in 50-60% of cases, the diseases were concurrent [17,19].

The presence of a link between COVID-19 and autoimmune diseases is evidenced by the international team of scientists [13], who report that COVID-19 is similar to autoimmune diseases in terms of clinical manifestations, immune responses and pathogenic mechanisms. Persistent immune responses are involved in the pathogenesis of both diseases [13]. Similar to systemic autoimmune diseases, COVID-19 can manifest itself with heterogeneous and systemic clinical manifestations [13], which make it difficult to diagnose new cases of MPA in patients experienced COVID-19. Moreover, Thu Aung Z and others [17], in turn, emphasise that COVID-19 and AAVs are multisystem diseases. It is believed that there is a causal link between both conditions, which is supported by reports of numerous clinical cases [8, 14, 16-22]. Therefore, physicians should be aware of the potential spectrum of systemic and autoimmune diseases that can be triggered by SARS-CoV-2 infection. This will provide a timely diagnosis and treatment initiation [10], which can save not only lives, but also protect other organs from damage. Moreover, COVID-19 pandemic has changed the approach of various clinicians to ANCA-related vasculitis [25]. For this reason, it is essential to consider the importance of a high suspicion index for AAV in people experienced COVID-19.

A team of experts from the UK, India, Kazakhstan and Ukraine, eight of the world's leading research centres developed online questionnaire with 28 questions based on relevant global practice guidelines and AAVs recommendations for the evaluation of the level of knowledge and perceptions of physicians regarding ANCA-associated vasculitis, as well as conducted online survey during COVID-19 pandemic [25]. The report on this issue was published on November 21, 2022, where 113 respondents from 21 countries indicated the need for enhanced medical education, which could increase

practitioners' awareness and knowledge regarding ANCA-associated vasculitis.

It is commonly known that timely recognition, rapid diagnosis and early initiation of active specific treatment for MPA remain one of the most challenging tasks in real clinical practice, as MPA is characterised by heterogeneous initial manifestations and variable clinical presentation. Given that MPA is a systemic vasculitis, the involvement of many organs can lead to a wide range of signs and symptoms [26-28]. The kidneys and lungs are the most typical organs involved in MPA. Initially nonspecific and then heterogeneous symptoms can mislead the diagnostic search and cause a significant delay in adequate treatment for AAV [27].

In 2020, a group of French researchers [29] published the data of a retrospective analysis of clinical presentation in 378 patients with MPA from the French Vasculitis Study Group Registry. At the time of diagnosis, the main clinical manifestations of MPA included: renal dysfunction (74%), arthralgia (45%), skin (41%) and lungs (40%) involvement, mononeuritis (32%), and less frequent alveolar haemorrhage (16%), cardiomyopathy (5%) and severe gastrointestinal signs (4%); mean serum creatinine was 217 $\mu\text{mol/l}$ [29].

Thus, MPA is a multisystem disease characterised by polymorphism nonspecific clinical signs and diverse clinical manifestations depending on the affected organs. It is worth noting that systemic necrotising vasculitis are great masqueraders, and sometimes their manifestations can often differ significantly from the typical recognised disease patterns, and therefore, received the descriptive name "the Great Masquerades" [30]. It is important to note that AAVs can also present with unusual manifestations of the disease [30], which can vary. In addition, the variability in clinical symptoms

may also be related to the existing comorbidity. From clinical perspective, comorbid pathology complicates the course of underlying disease and leads to changes in the usual clinical presentation. Moreover, existing comorbid conditions can mislead the doctor and direct the diagnostic search in the wrong direction, as the clinical case presented clearly demonstrates. Therefore, COVID-19 may trigger a new-onset MPA, especially in patients with predisposing factors. Thus, AAV should definitely be included in the differential diagnosis in patients with COVID-19 who present with polymorphic clinical manifestations.

CONCLUSIONS

During the COVID-19 pandemic, the early diagnosis of MPA for healthcare professionals of different specialties remains as challenging as it was before, since these two diseases are multisystem disorders sharing common pathogenetic mechanisms of development and clinical manifestations, as well as casual relationship between them.

Polymorphism of nonspecific clinical signs and multiorgan lesions are characteristic features of MPA which can vary from patient to patient, mislead physicians and leading to a misdirected diagnostic search. Rapid and early diagnosis of MPA is important for initiating therapy, which can save not only lives, but also protect other organs from damage.

Since MPA is a multidisciplinary problem, there is a need to increase the awareness and knowledge of practitioners of all specialties regarding AAVs and MPA in particular, as evidenced by the data of online survey conducted during the COVID-19 pandemic among doctors from 21 countries worldwide.

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