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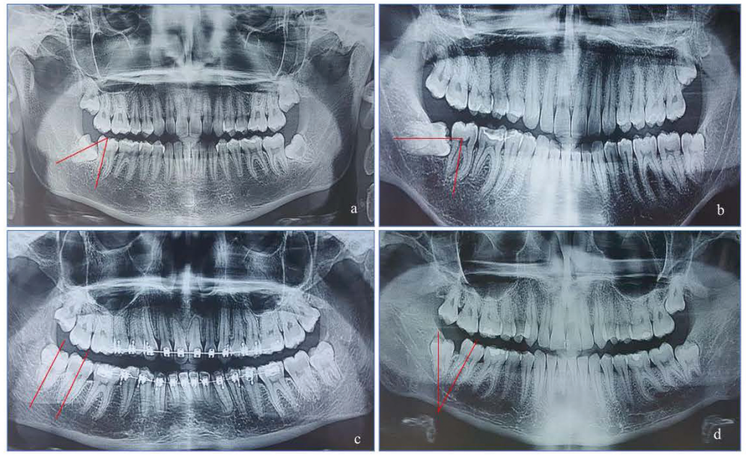
CONTENT HIGHLIGHTS:

Gabriela Motelica
Risk assessment of pericoronitis in correlation with the position of the inferior third molar.



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CONTENT

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BERLIN-CHEMIE MENARINI

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Editorial board

Iodură de potasiu

Iodomarin®

IODUL ESTE NECESAR PENTRU VIAȚĂ[1*]

SCHEMA DE ADMINISTRARE A PREPARATULUI IODOMARIN®[2,3]

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PROFILAXIA GUȘII[2,3]		
Iodomarin® 100 mcg	*câte ½ - 1 comprimat pe zi (50-100 mcg iod)*	*câte 1-2 comprimate pe zi (100-200 mcg iod)*
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Iodomarin® 200 mcg	*câte ½ - 1 comprimat pe zi (100-200 mcg iod)*	*câte ½ - 1 comprimat pe zi (100 -200 mcg iod)*

REZUMATUL CARACTERISTICILOR PRODUSULUI: DENUMIREA COMERCIALĂ A MEDICAMENTULUI: IODOMARIN 100, 100 mcg comprimate, IODOMARIN 200 200 microgame comprimate. **COMPOZIȚIA CALITATIVĂ ȘI CANTITATIVĂ .** 1 comprimat conține iodură de potasiu – 131 mcg (echivalent cu 100 mcg iod); 1 comprimat conține iodură de potasiu – 262 mcg (echivalent cu 200 mcg iod); **FORMA FARMACEUTICĂ.** Comprimate rotunde, plate, de culoare albă sau aproape albă, cu margini teșite și incizie pe una dintre suprafețe. Comprimatul poate fi divizat în două părți egale. **Indicații terapeutice Iodomarin 100:** Profilaxia tulburărilor datorate deficitului de iod (prevenirea gușii endemice sau după tratamentul chirurgical al gușii prin deficit de iod). Tratamentul gușii difuze cu eutiroidie, produsă prin deficit de iod, la nou-născuți,la copii, adolescenți și adulți tineri. **Doze și mod de administrare: Profilaxia gușii:** La un aport insuficient a iodului cu alimentele în conformitate cu normativele OMS aportul zilnic minim de iod pentru adulți constituie 150 – 300 mcg) adăugător se indică următoarele cantități de iod: *Nou-născuți și copii*: câte ½ - 1 comprimat Iodomarin 100 pe zi (echivalent cu 50-100 mcg iod). *Adolescenți și adulți*: câte 1-2 comprimate Iodomarin 100 pe zi (echivalent cu 100-200 mcg iod). *Sarcina și perioada de alăptare*: câte 2 comprimate Iodomarin 100 pe zi (echivalent cu 200 mcg iod). *Profilaxia recidivării gușii după tratamentuli chirurgical sau după finisarea tratamentului medicamentos al gușii cu hormoni tiroidieni*: câte 1-2 comprimate Iodomarin 100 pe zi (echivalent cu 100-200 mcg iod). *Adulți de vîrstă tînără*: câte 3-5 comprimate Iodomarin 100 pe zi (echivalent cu 300-500 mcg iod). Mod de administrare. Preparatul se administrează oral, după masă, cu o cantitate suficientă de apă. Durata tratamentului: Administrarea profilactică a Iodomarin 100 trebuie efectuată în decurs de câțiva ani, deseori – pe parcursul întregii vieți. Tratamentul gușii la nou-născuți se efectuează timp de 2-4 săptămâni, iar la copii, adolescenți și adulți – 6-12 luni. **Indicații terapeutice Iodomarin 200:** Profilaxia tulburărilor datorate deficitului de iod (prevenirea gușii endemice sau după tratamentul chirurgical al gușii prin deficit de iod). Tratamentul gușii difuze cu eutiroidie, produsă prin deficit de iod, la copii, adolescenți și adulți tineri. **Doze și mod de administrare: Profilaxia gușii:** La un aport insuficient a iodului cu alimentele (în conformitate cu normativele OMS aportul zilnic minim de iod pentru adulți constituie 150 – 300 mcg) adăugător se indică următoarele cantități de iod: *Nou-născuți și copii*: Pînă la ½ comprimat Iodomarin 200 pe zi (echivalent cu 100 mcg iod). *Adolescenți și adulți*: câte ½ - 1 comprimate Iodomarin 200 pe zi (echivalent cu 100-200 mcg iod). *Sarcina și perioada de alăptare*: câte 1 comprimat Iodomarin 200 pe zi (echivalent cu 200 mcg iod). *Profilaxia recidivării gușii după tratamentuli chirurgical sau după finisarea tratamentului medicamentos al gușii cu hormoni tiroidieni*: câte ½ - 1 comprimate Iodomarin 200 pe zi (echivalent cu 100-200 mcg iod). *Tratamentul gușii eutiroide: Nou-născuți, copii și adolescenți*: câte ½ - 1 comprimate Iodomarin 200 pe zi (echivalent cu 100-200 mcg iod). *Adulți de vîrstă tînără*: câte 1½-2 ½ comprimate Iodomarin 200 pe zi (echivalent cu 300-500 mcg iod). Mod de administrare: Preparatul se administrează intern, după masă, cu o cantitate suficientă de apă. Durata tratamentului: Administrarea profilactică a Iodomarin 200 trebuie efectuată în decurs de câțiva ani, deseori – pe parcursul întregii vieți. Tratamentul gușii la nou-născuți se efectuează timp de 2-4 săptămâni, iar la copii, adolescenți și adulți – 6-12 luni. **Contraindicații:** Hipersensibilitate la iodura de potasiu sau la oricare dintre excipienți. Hipertiroidism manifest; hipertiroidism latent în cazul administrării unor doze mai mari de 150 µg iod pe zi; Adenom toxic tiroidian, gușă nodulară la administrare în doze de 300-1000 mcg /zi), cu excepția pregătirii preoperatorii prin terapia cu iod după Plummer. **Atenționări și precauții speciale pentru utilizare:** Comprimatele Iodomarin 100 și 200 conțin lactoză. Pacienții cu afecțiuni ereditare rare de intoleranță la galactoză, deficit de lactază sau sindrom de malabsorbție la glucoză-galactoză nu trebuie să utilizeze acest medicament. **Reacții adverse:** Tulburări ale sistemului imunitar. *Foarte rare:* Reacții de hipersensibilitate - cum, de exemplu, rinita cauzată de iod, reacții cutanate iododerma buloasă sau tuberoasă, dermatită exfoliativă), edemul pielii sau mucoaselor edem angionevrotic), febră, acnee și edematierea glandelor salivare. Tulburări endocrine: *Foarte rare:* În cadrul terapiei gușii doza zilnică de 300-1000 mcg iod) în unele cazuri este posibilă dezvoltarea hipertiroidismului, provocat de iod. În marea majoritate a cazurilor drept premiză pentru acesta servește prezența porțiunilor limitate sau difuze autonome ale glandei tiroide. De obicei, riscului sunt supuși pacienții vârstnici, care suferă de gușă o perioadă îndelungată de timp. **DEȚINĂTORUL CERTIFICATULUI DE ÎNREGISTRARE:** Berlin-Chemie AG Glienicker Weg, 125 D-12489 Berlin, Germania. **DATA REVIZUIRII TEXTULUI:** Iulie 2018

** În caz de aport insuficient de iod.*

1. *WHO/UNICEF/ICCIDD. Assessment of iodine deficiency disorders and monitoring their elimination: a guide for programme managers, 3rd ed. Geneva: World Health Organization; 2007;*
2. *RCP Iodomarin 100, iulie 2018;* ***3.*** *RCP Iodomarin 200, iulie 2018.*

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BERLIN-CHEMIE MENARINI

EDITORIAL

The demographic crisis and medicine

Ion Dumbraveanu, MD, PhD,

Leading research project „Male infertility - systemogenesis of risk factors, study of pathological mechanisms and optimization of prevention, monitoring and treatment strategies in the population of the Republic of Moldova".

I think that few of those over 50, would have believed at the beginning of the 21st century, that in a short time we will end up living in a period of deep crises: the energy crisis, the food crisis, the financial crisis, the climate crisis, the crisis of the health, the demographic crisis, etc. However, some current crises were predictable. In the scientific and academic circles, predictions have been made and are being made that later come true. One of these concerns is referred to the worrisome demographic situation, especially in developed and developing countries. Although the Republic of Moldova is far from being a well-developed country from an economic and social point of view, it has a dramatic demographic situation with direct existential connotations, and I can say that we have entered and are deepening into an obvious demographic crisis. The population of the country in the last 2 decades decreased by about 1/3, from over 4 million inhabitants to 2 million 800 thousand, with tendencies to continue the negative trend with another 20-30% towards the year 2035.

The definition of the demographic crisis encompasses the notions of declining birth rates, increased mortality and accelerated ageing with the decrease in natural population growth, along with uncontrolled emigration. All these components are commonplace in the Republic of Moldova. Of course the state authorities are aware of the situation and are trying to manage it. But unfortunately, very often the demographic situation, as well as other society problems, have been approached by the ostrich method (hiding the head in the sand so that maybe the tail cannot be seen) or unilaterally, without a broad, interdisciplinary involvement, of all those on whom the finding of solutions depends. And while in recent years the seriousness of the problem and the involvement of international institutions which have funded the development of projects have highlighted the situation, the approach still raises many questions. I have carefully studied recent reports on the demographic situation in Moldova. They are very comprehensive and up-to-date from a statistical, economic and social point of view. At the same time, the medical aspects of the problem are addressed briefly and somewhat unilaterally. Some timid solutions are also proposed, in various dimensions, but without a clear, multidimensional, state-level strategy for redressing the situation. I agree that the economic, financial and social aspects are very important in managing a demographic crisis, but they are not the only ones. The examples of France, Italy, and Spain, countries that are economically overdeveloped but with clearly negative demographic trends are eloquent. The improvement of the economic situation will contribute to the migration aspect of the problem. Other mechanisms, including medical and psycho-social ones, need to be put in place to solve the problem. Birth rate and fertility rates are the cornerstone of demography. Population numbers can only be increased by increasing the number of births. Then comes health maintenance, health being the main factor in social and economic development. There can be no social evolution without human beings, just as there can be no proper development when human beings are sick. Increasing life expectancy, reducing morbidity and mortality are important aspects because they can increase demographic indices, factors following birth. At the same time, social and economic factors influence the state of a country's sanitary system and directly affect health. Most medical demographic studies, until relatively recent ones, have paid more attention to mortality than to birth rates. It was and it is a salutary method of maintaining the health of the existing population. Most strategies in sexual and reproductive health were and are focused on the prevention of sexually transmitted infections, and in family planning on the use of contraception or safe abortion. This has been and still is the society demand. This is acceptable, but not enough. Everyday reality and my own medical experience over 30 years show other trends. About 15-20% couples or families cannot have children, or have fewer than they would want. The causes, mostly medical, are evenly divided between men and women. There are increasingly paradoxical situations when the couple uses contraceptive methods, including hormonal ones, for many years, and later it is found that they are in fact infertile. Therefore, there are situations of prescribing and using contraceptives without a basic evaluation, one of the causes being the small number of medical offices and specialists in the field or the lack of access to quality medical services. Unfortunately, in most sexual-reproductive health policies the focus is only on women's health and only in the last decade in some countries are state doctrines and policies emerging for men. The average time from the detection of the problem to the visit to an andrologist in the Republic of Moldova is about 1.5-2 years. The state sector in the field of sexual and reproductive health is practically absent. In the private sector services are expensive and there are often doubts about their quality. There are many gaps in the sexuality education system,

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and the younger generation is educated with the idea that sexual activity is mostly for pleasure and can start early, pregnancy must and can be prevented, sexually transmitted diseases can be avoided by using condoms, and planning the birth of their own children is somewhere in the very distant future, if at all. This doctrine has positive sides, by reducing the frequency of sexually transmitted diseases (not the papilloma virus) and the number of abortions, but at the same time it leads to an increase in the level of promiscuity (a large number of sexual partners), the decrease to complete disappearance of the phases a sexual act and the importance of a heterosexual family. This leads to the fact that people focus more on themselves, increase in the number of divorces and the tendency to have the first child after the age of 40.

A new approach to demographic policies at national level is obvious and imperative, with direct coordination of state bodies and the involvement of all those who can change the situation, not only formally but in reality. The first concrete steps that can be taken are to increase access to quality reproductive health services for both women and men. The Ministry of Health and the Nicolae Testemitanu SUMPh can get involved by training medical staff and carrying out scientific research, the National Health Insurance Company by funding programs for the diagnosis and treatment of infertile couples, including invitro fertilization, the Ministry of Labor and Social Protection by creating appropriate instruments to support young mothers and children, etc... In addition, of course, national and international financial institutions by providing real funding according to needs and not through derisory allocations.

We must remember! As long as the focus of any demographic approach is not on people and human health, any population growth initiative is doomed to failure.

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RESEARCH ARTICLE

Changes of oxidative stress indices and antioxidant system in the liver tissue on the administration of some coordination compound of copper, derivatives of thiosemicarbazide

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What is not yet known on the issue addressed in the submitted manuscript

The biochemical action mechanisms of a some new copper coordination compound, derivatives of thiosemicarbazide (CC), in particular, on the peroxidative processes and the level of antioxidant system in liver tissue have not yet been studied.

The research hypothesis

CC could be exerting a significant influence on the metabolic processes in the liver tissue, in particular on free radical oxidative processes and on the antioxidant system.

The novelty added by manuscript to the already published scientific literature

The influence of new CC on free radical oxidation processes and antioxidant system has been elucidated in liver tissue, estimate, and select the most informational biomarkers for the assessment of oxidative stress in liver tissue and which can be used to determine the effectiveness of new local remedies.

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Abstract

Introduction. Identification, study and testing of new remedies for treatment approaches of diseases, resulting from imbalance between oxidants and antioxidants in favor of oxidants, with potentially destructive potential and pathogenesis in liver disorders is of particular interest due to the increase in incidence and severity of these pathologies.

Material and methods. The action of novel local copper coordination compounds, thiosemicarbazide derivatives - CMD-4, CMJ-33 and CMT-67, was evaluated in experiments on white rats after subcutaneous administration in two different doses (0.1 and 1.0 μM/kg) 3 times a week for 30 days. The main indices of oxidative stress were evaluated: the level of malonic dialdehyde (*MDA*), nitric oxide derivatives (*NO*), S-nitrosothiols, advanced glycation end products (*AGEs*), advanced oxidation protein products (*AOPP*) and ischemia-modified proteins (*IMP*), and antioxidant system: - superoxidismutase (*SOD*) and catalase activity *(CAT)*, the level of histidine (*His*) and total antioxidant activity (*TAA*) in liver tissue of white rats.

Results. The administration of CC resulted in the reduction of oxidative stress indices - *MDA*, *AGEs* and *S-nitrosothiols*, which denotes the antioxidant effect of the studied compounds. The level of *NO* and *AOPP* derivatives does not change substantially. When administering CMD-4 (1 μM/kg), *SOD* activity and catalase function decreased markedly. Changes in the content of *His* and *TAA* have been shown to be inconclusive, maintaining within the limits of the values recorded in the control group.

Conclusions. The elucidation of the modifications of the free radicals processes in liver tissues, which are the basis of the *CC* action, broadens the theoretical knowledge about the biological properties of a number of chemical compounds; as well provide new possibilities to explore perspective objects in order to obtain new efficient drug preparations.

Keywords. Oxidative stress, antioxidant system, liver tissue, coordination compounds of copper.

Introduction

Studies in recent years have brought more and more evidence, such as that damage to cells by reactive oxygen species *(ROS)* and reactive nitrogen species *(RNS)* are the most important factors leading to aging and degenerative diseases, such as cancer, cardiovascular and liver diseases, cataracts, chronic inflammatory processes, renal insufficiency and so on [1].

Identification, study and testing of new remedies for treatment approaches of diseases, resulting from imbalance

between oxidants and antioxidants in favor of oxidants, with potentially destructive potential and pathogenesis in liver disorders is of particular interest due to the increase in incidence and severity of these pathologies.

Thus, it is certain the need to develop new compounds, which could serve as a basis for the development of drug preparations for the prevention and treatment of the above mentioned diseases, including liver diseases.

In this respect, derivatives of thiosemicarbazides are of particular interest, which could have a significant influence on metabolic processes.

Research carried out over the last decade has shown their therapeutic efficiency and perspective of valorification as raw material for obtaining medicinal remedies [2-5].

At the same time, their biochemical action mechanisms, in particular, on the peroxidative processes in the liver tissue, are not known in detail.

The aim of this study is to research the influence of new local copper coordination compounds, derivatives of thiosemicarbazides on free radical oxidation processes and antioxidant system in liver tissue, estimation, and selection of the most informative biomarkers for assessing the level of oxidative stress and which can be used to determine the effectiveness of new local remedies.

Material and methods

Study design

The study is preclinical experimental. The research was approved by the Research Ethics Committee of the *Nicolae Testemitanu* State University of Medicine and Pharmacy (favorable minute no. 73 from 26.04.2017).

Animals included in the study

In this study have been used new local copper coordination compounds, derivatives of thiosemicarbazides (CC) – 4 – ethyl – 2 - [phenyl (pyridine – 2 - yl) methylidene] hydrazine – 1 - carbothioamide (CMD-4) , chloro - {4 - (3 - methoxyphenyl) – 2 - [1 - (pyridine – 2 - yl) ethylidene] hydrazine – 1 - carbothioamide} copper (CMJ-33) and nitrato - {N – phenyl – N' - (pyridine - 2 - ylmethylidene) carbamohydrazonothioato} copper (CMT-67) synthesized in the Laboratory of Advanced Materials in Biopharmaceuticals and Technics at Moldova State University [5]. The autochthonous *CC* action on the liver tissue has been evaluated in experiments on a sample of 46 Wistar line male rats with 180 – 250 g mass, divided into 6 groups of 7-8 animals in each.

The first group – control, was made up of 8 intact animals, maintained at a normal diet of vivarium and whom was administered subcutaneous three times per week for 30 days physiological solution. Animals on the experimental groups 2 - 6 were administered the subcutaneous *CC* 3 times a week for 30 days during 30 days in the following sequence: 2nd group - CMD-4 (0.1 μM/kg body weight), 3rd group - CMD-4 (1.0 μM/kg body weight), 4th group - CMJ-33 (0.1 μM/kg body weight), 5th group - CMJ-33 (1.0 μM/kg body weight) and 6th group CMT-67 (0.1 μM/kg body weight).

Method of processing liver tissue

24 hours after the last administration of local *CC*, the animals were sacrificed under mild narcosis with sulfuric ether and the liver was collected. All operations were performed in glacial environment. The preparation of the material for the determination of the biochemical indices has been carried out as follows. The phosphate buffer solution 0.1 M (pH 7.4) containing 1 mm EDTA has been used as the dispersion medium, so that the final dilution of the homogenate constitutes 1:10. For complete destruction of the cell membranes the homogenate was processed with triton X-100 in the final concentration 0.1%. Subsequently, the tissue homogenates were subjected to centrifugation for 15 minutes at 5000 rpm, and the supernatant was transferred to clean tubes and until examined kept in the freezer at minus 40°C. The entire process of preparing tissue homogenates is performed under regulatory conditions for the assessment of biochemical parameters.

Tests of oxidative stress and antioxidant system tested

The intensity of oxidative stress was evaluated by determining the following laboratory parameters: malonic dialdehyde *(MDA)*, nitric oxide *(NO)* derivatives, S-nitrosothiols *(RSNO)*, advanced glycation end products *(AGEs),* advanced oxidation protein products *(AOPP)* and ischemia-modified proteins *(IMP)*.

The changes in the antioxidant protection indices were evaluated by determination of the activity of superoxidismutase *(SOD),* catalase *(CAT)*, histidine *(His)*, as well as the level of total antioxidant activity that is based on the antioxidants inhibition of the absorption of the 2.2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation *(ABTS).*

The assessment of oxidative stress and antioxidant system was performed according to the procedures described previously [6].

Statistical processing method

The statistical evaluation of the obtained data performed with use of the computer program StatsDirect. The arithmetic mean ± error of the mean (M ± m) was calculated. The nonparametric statistical test „U Mann-Whitney" and the significance threshold „p" ($p < 0.05$) were used to test the significant difference between the studied indices of the compared groups.

Results

The evaluation results of lipid peroxide indices: *MDA, NO, AGEs, IMP, AOPP,* and *RSNO* in liver tissue when administering local *CC* are reflected in the statistical data of Table 1.

The study shows that *MDA* decreases statistical suggestive in the liver tissue by 14% - 40% under the influence of most of the studied preparations, except only the CMJ-33 compound (0.1 μM/kg), which shows a discrete tendency to decrease. Changes in the level of *NO* derivatives in liver tissue under the action of tested *CC* proved to be inconclusive. Administration of the tested *CC* results in a discrete reduction in the level of S-nitrosothiols in the liver, with

Table 1. *Influence of local CC on the lipid peroxidation indices in the liver tissue.*

Study groups	MDA nM/g.tissue	NO µM/g.tissue	RSNO nM/g.prot.	AGEs µg/g.prot.	AOPP nM/g.prot.	IMP µM/g.prot.
Control	112.5±5.5 (100%)	4.6±0.4 (100%)	73.7±4.6 (100%)	81.6±5.3 (100%)	60.4±4.2 (100%)	182.6±8.0 (100%)
CMD-4 0.1 µM/kg	95.8±5.6* (85%)	3.8±0,2 (83%)	68.6±4.6 (93%)	70.5±9.0 (86%)	65.2±6.4 (108%)	131.3±15.0* (72%)
CMD-4 1 µM/kg	96.6±4.5* (86%)	4.0±0.3 (88%)	61.8±2.0* (84%)	60.2±4.5** (74%)	63.6±6.0 (105%)	156.4±14.5 (86%)
CMJ-33 0.1 µM/kg	89.9±7.8 (80%)	4.4±0.4 (95%)	62.4±4.7 (85%)	67.7±4.1 (83%)	66.9±7.1 (111%)	159.5±14.2 (87%)
CMJ-33 1 µM/kg	90.4±4.0* (80%)	4.3±0.2 (93%)	58.7±2.8* (80%)	65.5±5.6* (80%)	66.1±5.0 (109%)	166.4±6.2 (91%)
CMT-67 0.1µM/kg	67.2±4.6** (60%)	3.8±0.3 (81%)	63.5±2.8 (86%)	71.9±6.7 (88%)	64.8±10.0 (107%)	190.5±23.5 (104%)

*Note:** - statistically significant difference with the control group (*-p<0.05; ** - p<0.01; *** - p<0.001); local CC - copper coordination compounds, derivatives of thiosemicarbazides; CMD-4 - 4- ethyl – 2 - [phenyl (pyridine – 2 - yl) methylidene] hydrazine – 1 - carbothioamide; CMJ-33 - chloro - {4 - (3 - methoxyphenyl) – 2 - [1 - (pyridine – 2 - yl) ethylidene] hydrazine – 1 - carbothioamide}copper; CMT-67 – nitrato - {N - phenyl – N '- (pyridine – 2 - ylmethylidene) carbamohydrazonothioato} copper; MDA - malonic dialdehyde ; NO - nitric oxide derivatives; RSNO - S-nitrosothiols; AGEs - advanced glycation end products ; AOPP - advanced oxidation protein products; IMP - ischemia-modified proteins.

the exception of the compounds CMD-4 and CMJ-33, which at maximum doses (1.0 µM/kg) have the property to veraciously decrease the level of *S- nitrosothiols* by 16% and 20% ($p < 0.05$) respectively compared to the control group. There was a discrete tendency to decrease the intensity of the *AGEs* product formation processes in the liver of white rats after the administration of the tested bioactive compounds. At the same time, compounds CMD-4 and CMJ-33 at the maximum dose of 1.0 µM/kg statistically significantly reduce the level of *AGEs* by 20% and 26% compared to the values attested in the control group.

Changes in the values of advanced oxidation protein products — *AOPP* in the liver were found to be without statistical relevance, these values remaining at the level of the reference parameters.

The results of the study show that the tested *CC* did not induce statistical relevance changes in the *IMP* content in the liver, except for the compound CMD-4 (0.1 µM/kg), which reduces this index by 28% compared to the control values.

The evaluation results of the antioxidant protection indexes: *SOD, CAT, His* and *TAA* in hepatic tissue on the action of local *CC* are reflected in the statistical data of Table 2.

Table 2. *Influence of local CC on the antioxidant protection indices in the liver tissue.*

Study groups	SOD u/g.prot.	CAT µM/s.g.prot.	His µM/g.prot	TAA nM/g.prot.
Control	23.0±0.9 (100%)	66.9±5.2 (100%)	6.72±0.4 (100%)	2.36±0.04 (100%)
CMD-4 0.1 µM/kg	21.1±1.03 (92%)	48.0±9.1 (72%)	7.46±0.2 (111%)	2.38±0.03 (101%)
CMD-4 1 µM/kg	17.6±0.8* (76%)	62.8±3.4 (94%)	7.14±0.1 (106%)	2.23±0.02 (94%)
CMJ-33 0.1 µM/kg	26.5±2.2 (115%)	60.2±3.4 (90%)	6.49±0.2 (97%)	2.29±0.03 (97%)
CMJ-33 1 µM/kg	26.7±1.15 (116%)	51,.5±2.1* (77%)	6.98±0.1 (104%)	2.35±0.04 (100%)
CMT-67 0.1 µM/kg	24.5±2.4 (106%)	53.4±3.1 (80%)	7.28±0.3 (108%)	2.37±0.04 (100%)

*Note:** - statistically significant difference with the control group (*-p<0.05; ** - p<0.01; *** - p<0.001); local CC - copper coordination compounds, derivatives of thiosemicarbazides; CMD-4 – 4 - ethyl – 2 - [phenyl (pyridine – 2 - yl) methylidene] hydrazine – 1 - carbothioamide; CMJ-33 - chloro - {4 - (3 - methoxyphenyl) – 2 - [1 - (pyridine - 2 - yl) ethylidene] hydrazine – 1 - carbothioamide} copper; CMT-67 - nitrato - {N – phenyl - N' - (pyridine – 2 - ylmethylidene) carbamohydrazonothioato} copper; SOD - superoxidismutase; CAT - catalase activity; His - histidine; TAA - total antioxidant activity.

The study shows that *SOD* decreases statistically conclusively by 24% ($p < 0.05$) when administering CMD-4 (1.0 µM/kg), and the catalase function in this case is veracious decreasing by 23%. The changes in the content of *His* and *TAA* proved to be inconclusive, keeping them within the limits of the values recorded in the control.

Discussion

In this study, the level of oxidative stress in the liver tissue of the laboratory animals subjected to the action of local CC, derivatives of thiosemicarbazide, was analyzed. It is evident the selective action of the studied compounds on the indices of oxidative stress and antioxidant system, which depends

on the degree of their employment at different stages of the metabolic processes that occur in the liver tissue.

As free radicals have very short half-life periods, the *in vivo* assessment of oxidative stress is based on the measurement of different stable oxidized products of biomolecules in the cells and tissues of living organisms. Oxidative stress assessment methods are often referred to as fingerprinting methods, by which end products resulting from the interaction of *ROS* and *RNS* with various biomolecules such as membrane lipids, proteins and amino acids, carbohydrates, nitrogenous bases, etc., are measured [7].

Primary products of the peroxidation of polyunsaturated fatty acids result in the formation of peroxyl and alkoxyl radicals, which are very reactive and have a short life, being further subjected to other reactions with the formation of different aldehydes, such as *MDA*, 4-hydroxyalkenals and acrolein. These aldehydes, because of their electrophilic nature, have a very high damage potential. The most abundant aldehyde resulting from the lipids peroxidation is the *MDA* and its accumulation may cause alterations in proteins, nucleic acids and many other biomolecules.

In this study we obtained significantly lower levels of the end product of lipid peroxidation - *MDA* in liver tissue under the influence of most of the preparations studied, except only CMJ-33 (0,1 μM / kg), which shows a discreet tendency to decrease. Reducing the level of *MDA* under the influence of most of the tested *CC* in liver tissue, possibly, is an expression of several factors: the intensity of the formation of the primary products of lipid peroxidation, the biosynthesis of *MDA*, the speed of the metabolic processes in the tissue and the ability of the body to eliminate this final product, as well as the level of substances with antioxidant role. We can admit that the tested compounds, due to their property of lowering the level of *MDA* by various mechanisms, can increase the efficiency of cellular protection against various peroxidants and cytotoxic agents.

Therefore, the hepatic level of *MDA* could be used as a test to assess the efficacy of tissue protection against lipid peroxidation by free radicals when testing different *CC*.

NO is a free radical that plays an important role in various physiological processes, and *RSNO* represent the natural reservoir (deposit) and form of transport of nitric oxide. Nitric oxide due to its high reactivity can cause S-nitrosylation of cysteine residues from proteins or peptides, which is an important redox signaling mechanism that regulates a wide range of biological, physiological and cellular functions in various tissues, including liver [8].

The tendency to decrease *NO* concentration, recorded in our research, even if it did not reach the statistical significance limit, is probably due to the inhibition by tested compounds of nitric oxide synthase (enzyme that increases the level of *NO* in tissues) and could be explained by the antitumor activity of these compounds [4-5]. These findings are consistent with the data of some authors, who found that nitric oxide synthase inhibitors that reduce *NO* production might have a therapeutic role in certain cancers due to their property of reducing angiogenesis, proliferation, and causes suppression of tumor invasion [9].

The decrease in the level of *RSNO* in the liver when administering the maximum doses (1.0 μM / kg) of the compounds CMD-4 and CMJ-33, recorded in our experiences is probably due to their intense decomposition by the transition metal ions, such as copper (II) ions, which are part of the tested compounds, and by various redox active species, including reactive oxygen species [10], or by catalytic denitrosylation by specific enzymes, such as thioredoxin (Trx) and S-nitrozoglutation reductase *(GSNOR*) - enzymes that eliminate *NO* from nitrosylated proteins / peptides [11].

This could create a deficiency of *NO* signaling, which would decrease the proliferation intensity of the liver progenitor cells and cause the reduction of the processes of restoration in the hepatic lesions and, it is not excluded, could cause manifestations of liver toxicity. Indeed, according to some researchers, the increased *NO* and *RSNO* levels, in contrast to the low levels, induce the proliferation of liver progenitor cells and improve the liver restoration from partial hepatectomy [12].

In view of these aspects, it is important for the research of new bioactive compounds to evaluate the NO/RSNO system in order to identify potential changes of this system and to provide a better understanding of the mechanisms of action of these compounds, which will facilitate not only the discovery of new targets for the action of bioactive compounds, but also the development of new therapeutic agents.

Studies carried out over the last decades have brought more and more evidence that during the glycation process, namely the interaction of carbohydrates with the free amine groups of proteins, the early glycation products are formed first, and they are subsequently rearranged into final structures, called advanced glycation end products - *AGEs* through a series of complex chemical reactions. *AGEs* are involved in the pathogenesis of atherosclerosis, chronic inflammatory processes, aging, cancer, neurodegenerative diseases, such as Alzheimer's disease, etc. [13].

The obtained results indicated the discrete tendency to decrease the intensity of the *AGEs* product formation processes, and the compounds CMD-4 and CMJ-33 at the maximum dose of 1 μM / kg significantly decrease the level of AGEs in the liver tissue, compared to the values attested in the control group. This fact indicates the anti-*AGEs* activity, and capacity to prevent the formation of *AGEs*, exercised by studied *CC*, and can be qualified as a positive moment, because, *AGEs* can interact with the specific receptors of the cell surface - *RAGE* (receptor for advanced glycation end products), which in the liver are expressed in hepatic stellate cells and myofibroblasts - cells involved in the fibrogenesis of liver disease, and therefore, they may alter intracellular cell signaling, gene expression, enhance *ROS* production and activation of several inflammatory pathways, including the release of pro-inflammatory cytokines, growth factors and adhesion molecules by activating the *NF*-κB (nuclear factor κappa-light-chain-enhancer of activated B cells) pathway [14].

Therefore, the level of *AGEs* can serve as a valuable indicator when evaluating the action of new *CC* on cells and tissues, including the liver.

As is known, proteins by their complex structure, present numerous points where they can suffer oxidative attacks, making them one of the first targets of free radicals.

The oxidative changes of proteins, called advanced oxidation protein products - *AOPP*, are protein products that contain dityrosine cross-linking bonds which can lead to various important functional consequences such as inactivation of enzymes, increased susceptibility to aggregation and resistance upon proteolysis, increased formation of free radicals, activation of the nuclear factor *NF*-κB and release of proinflammatory cytokines, adhesion molecules and growth factors [15, 16].

For that reason, it is considered that *AOPP* may be a more accurate biomarker of oxidative stress than lipid peroxidation products [16-18].

In this study the changes in the values of the advanced oxidation protein products - *AOPP* in the liver, proved to be statistically unsuggestive, they keeping within the limits of the reference parameters, so the studied compounds do not cause excessive synthesis of FR with prooxidative character at the local tissue level and, it is not excluded that they, on the contrary, annihilate the *FR* action due to their antioxidant properties. This fact is in agreement with the data of researchers who have detected antioxidant properties characteristic of thiosemicarbazonic compounds [19].

As is known in hypoxic and ischemic conditions in blood serum increases the concentration of ischemia modified albumin *(IMA)* increases, characterized by a significant change in the ability to bind the transition metals, especially cobalt [20]. The *IMA* level may increase in certain circumstances, such as myocardial ischemia, malignant neoformations, chronic diseases, and inflammatory processes [21, 22].

In view of this, we investigated the influence of *CC* on the content of *IMP* in liver tissue. The results of the study show that the tested *CC* did not induce statistically significant changes in the *IMP* content in the liver tissue, except for the CMD-4 (0.1 μM/kg) compound, which statistical truthful reduces this index to the control values, suggesting that this compound has the property of diminishing tissue hypoxia.

Thus, the results obtained demonstrate that the tested *CC* does not cause oxidative damage to the proteins of the liver tissue, which is manifested by the discrete tendency to decrease the intensity of the *AGEs* product formation processes under the influence of the majority of the investigated *CC* and to maintain the *PPOA* values within the normal values.

The conclusive reduction of the content of *AGEs* and *IMP* by the compound CMD-4 and *AGEs* by the CMJ-33 in the maximum dose of 1 μM/kg denotes their antioxidant effect.

Under the action of local *CC* studied the indices of antioxidant protection: *SOD, catalase, His* and *TAA* in the liver tissue of laboratory animals undergo changes of different intensity. Decrease of the activity of antioxidant enzymatic links *SOD* - enzymes that destroy superoxide anion O_2^- with the formation of O_2 and hydrogen peroxide), catalase (which inactivates H_2O_2) in the liver after administration of compounds CMD-4, CMJ-33 and CMT-67 can result with weakening of the cells enzymatic protection against the oxidation reactions with free radicals under conditions of a pronounced activation of this process. Not coincidentally, these two enzymes have the highest reaction rates (about 2 x 10^9 M-1 s-1), which allows them not only maximum efficiency, but also the possibility to act independently by providing substrates or coenzymes [23].

On the other hand, changes in the content of *His* (which has relevant antioxidant effects [24-25] and *TAA* (which includes various antioxidants, both enzymatic and non-enzymatic) have been shown to be inconclusive, maintaining them within the limits of the control values. This demonstrates that the studied compounds possess the ability to effectively remove free radicals (*FR)* and maintain the redox balance at local liver tissue level.

The antioxidant system, whereby aggressive compounds formed by the action of *FR* are converted into non-active compounds, play a decisive role in cell defending against the harmful action of *FR*. This system comprises a large number of elements, being as diverse as free radicals, cells containing a variety of scavenging-capacity substances for multiple radical species precisely to ensure maximum protection [26]. The high efficiency of antioxidants is explained by their wide diversity, the location in different cell compartments, the synergistic nature of their action, the summation of their combined action, each acting according to different mechanisms and varying levels of the chain of *FR* evolution in the body. Under normal conditions, antioxidants eliminate pro-oxidants, but under oxidative conditions, pro-oxidants predominate over antioxidants, which can lead to many inflammatory diseases, including cancer [27-28].

For these reasons, indices of antioxidant protection may be useful as diagnostic markers or therapeutic targets.

Because excessive *FR* formation can cause multiple cellular and tissue damage, their effects can be analyzed by local tissue markers of oxidative stress and antioxidant system. Further studies are needed to confirm the therapeutic utility of these bioactive tested compounds.

Conclusions

The most informative biomarkers of functionality of the prooxidant and antioxidant system have been estimated and selected to assess the level of oxidative stress in liver tissue to experimental exposure through the administration of local *CC* to laboratory animals and which can be used to determine the efficiency of new local drug preparations.

The reduction of oxidative stress indices (*MDA, AGEs, S-nitrosothiols, IMP*) when using local *CC* denotes their antioxidant effect.

The elucidation of molecular mechanisms who stay on the basis of the *CC* action expand the theoretical knowledge of the biological properties of a range of chemical compounds and also offers new possibilities to explore prospective objects for the purpose of obtaining new efficient drug preparations.

Declaration of conflicting interests

The authors declare no conflicts of interest.

Authors` contribution

The authors have equally contributed to the manuscript drafting, design and paper editing. All authors approved the final version of manuscript.

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RESEARCH ARTICLE

Features of lipid metabolism in membranes of rat cells at experimental traumatic brain injury on the background of chronic alcohol intoxication

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What is not yet known on the issue addressed in the submitted manuscript

Features of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex of rats with traumatic brain injury on the background of chronic alcohol intoxication.

The research hypothesis

Study of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex in experimental traumatic brain injury on the background of alcohol intoxication.

The novelty added by manuscript to the already published scientific literature

In experimental traumatic brain injury on the background of chronic alcohol intoxication significant and more pronounced changes in the content of total phospholipids, total cholesterol, their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex were revealed than in separately reproduced pathologies, as well as discoordination of the metabolism of individual fractions of phospholipids in membranes erythrocytes. The revealed pathophysiological features will make it possible to search for new remedy of pharmacological correction of targeted action.

Abstract

Introduction. Alcohol intoxication is often the cause of traumatic brain injury. The purpose of the work was to study the features of lipid metabolism in erythrocytes and mitochondria of the cerebral cortex cell membranes of rats in traumatic brain injury on the background of chronic alcohol intoxication.

Materials and methods. The studies were carried out on 140 male rats of the Wistar line weighing 170-250 g. Chronic alcohol intoxication was caused by using 15% ethanol as the only source of fluid for 20 days. Reproduction of traumatic brain injury was carried out by a shock model. Statistical analysis of data was conducted by using „Primer Biostatistics 6.0". The criteria of parametric statistics were used. The level of $p < 0.05$ was statistically significant.

Results. Chronic alcohol intoxication in rats and inducing of traumatic brain injury separately causes a significant change of total phospholipids and cholesterol content, and their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex. Particularly pronounced were the changes in studied objects with combined pathology, meaning, traumatic brain injury on the background of chronic alcohol intoxication.

Discoordination of lipid metabolism was unidirectional, manifested in a significant reduction in the content of total phospholipids, and increased total cholesterol, which led to a violation of the molar ratio of total cholesterol/phospholipids in the direction of increasing the coefficient, which is normally about 1.0.

In subsequent monitoring periods, there was a gradual restoration of individual fractions of phospholipids, but on the 30th day of the study, it did not reach the control values. That means that discoordination of the metabolism of individual phospholipid fractions was so pronounced, that even on 30th day of arbitrary reproduction did not reach the initial values, which again emphasizes the severity of the morphofunctional state of red blood cells membranes' disturbance.

Conclusions. The revealed features of lipid metabolism in the membranes of erythrocytes and mitochondria of the cerebral cortex in traumatic brain injury on the background of chronic alcohol intoxication are an important component for understanding the ongoing pathophysiological processes and searching for new effective drugs with targeted action.

Key words: membranes, erythrocyte, mitochondria of the cerebral cortex, alcohol intoxication, traumatic brain injury, lipids, phospholipids.

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Introduction

Chronic alcoholic intoxication for most countries of the world is a topical social and economic problem, the global manifestations of which are the appearance of a number of serious diseases and their complications, persistent loss of function and early disability. It is difficult to find organs and tissues in human body that would not be involved in damaged in chronic alcohol poisoning, in case of excessive alcohol abuse. The cardiovascular system, the pancreas, muscles, the immune system, and others are sensitive to an alcohol abuse damage. Especially high sensitivity to alcohol exhibits the liver, where its main metabolism occurs, with the formation of a highly toxic intermediate product, which is acetaldehyde [1]. There is no doubt that the most sensitive to the action of alcohol are central and peripheral nervous system, which results in systemic polyneuritis with functional impairment and pain manifestations and alcoholic encephalopathy [2].

Fundamentally in the multitropic damaging effect of alcohol is the initiation of a free radical chain of oxidation with the formation of partially oxidized products, which are aldehydes and ketones [3]. They cause morphofunctional changes in cellular membranes in the body, which consist of polymerizing effects on the protein component of the membranes with violation function of the phospholipid bilayer. Intensification of lipids peroxidation processes under the influence of toxic factors and, first of all, alcohol, is universal, nonspecific. Features of the structure and function of the central nervous system to a large extent due to the enormous variety of their lipid components, their properties, localization and metabolism [3]. This concerns both the content of total cholesterol (CE) and total phospholipids (PL), their molar ratio. The main structural unit of cellular lipids are PL, which represent a large family of phosphorus-containing natural lipids. Phospholipids form the main component of cell membranes and directly participate in the basic metabolic processes of the living cell. The most important function of PL is structural, since they form the phospholipid bilayer of all cell membranes. The composition of PL and their placement in membranes largely determines barrier properties of membranes, their permeability for various exo- and endogenous substances, ultimately their functional capacity [4]. Damage of the cell membranes, including neurons, consists of their destruction, which leads to a violation of the permeability for ions. In recent years established the undoubted role of erythrocytes in the regulation of various metabolic processes, both in normal and in the pathological conditions [5, 6]. It is also important that erythrocytes are the most accessible object for research, especially in clinical practice for assessing the patient's condition, the course of the disease and as a criterion for the effectiveness of the prescribed pharmacotherapy [7-9]. At the same time, practically there is no data about the influence of alcohol intoxication on the structural components of membranes of red blood cells. Often, alcohol intoxication is the cause of general injury and traumatic brain injury (TBI) is not the least. By itself, TBI causes a number of injuries both in the whole organism and in the brain. In the first place, this is an ischemic injury of cells that induces rigid hypoxia, which develops as a result of a violation of the morphofunctional state of cell membranes [9]. For normal functioning of the cell, lipids of the membranes must be in constant motion. Immobilization of lipids, which occurs as a result of TBI, alcohol intoxication, is associated with a qualitative rearrangement of the fatty acid pool and leads to a change in the lipid enzymes envelope and, consequently, to dysfunction of the cell [1, 8, 9]. In this regard, the maintenance of a stable structure and integrity of cell membranes is largely depends on the adaptive reorganization of the composition and the quantitative characterization of the cellular lipids bilayer [10]. Therefore, the studying of discoordination of lipid metabolism of cell membranes is a necessary step for the search and creation of a targeted action of drugs for the treatment of TBI on the background of chronic alcohol intoxication.

The purpose of the work – studying the features of lipid metabolism in membranes of erythrocytes and mitochondria of the cerebral cortex of rats at the traumatic brain injury on the background of alcohol intoxication.

Materials and methods of research

The research was conducted on 140 male rats of the Wistar line weighing 170-250 g at the age of 6 months from birth in the conditions of a chronic experiment in accordance with the requirements of the commission on bioethics of Odessa National Medical University. Animals were divided into four groups with 35 rats in each. In turn, each group were divided into 5 subgroups with 7 animals. The first subgroup served as control, the second – reproduced the experimental pathology, the third, fourth and fifth traced the restoration of the studied indicators in time intervals after 10, 20 and 30 days.

Chronic alcoholic intoxication, which was accompanied by the formation of alcohol addiction, caused by 20-days experiment in the „benefits of ethanol" test [11]. The animals were placed in individual boxes in which they had access to two drinking bowls: one with pure water and the second with 15 % ethanol. After measuring the volume of the drawn liquid, the coefficient of the alcohol preferences (K_n) was calculated: $K_n = 100\ \% \cdot V_{alcohol}/V_{general}$, where $V_{alcohol}$ is the volume of alcohol consumed, $V_{general}$ is the total volume of liquid drunk. The criterion for selecting animals for the subsequent experiment was the triad: drinking behavior; the preference for ethanol (with K_n not less than 50%) and the severity of neurological symptoms (by open field test and rotator test) [11]. The traumatic brain injury was reproduced by drawing a single blow to the crown of the head – the occipital part of the brain with a weight of 100 g, falling from a height of 80 cm, what is called the shock model [12].

In lipid extracts of erythrocytes the content of total CE and total PL in mmol/l were determined and also was calculated their molar ratio, which expressed by the coefficient of CE/PL. Blood was obtained from the caudal vein of rats. In parallel, in the lipid extracts of the membranes of the cerebral mitochondria, which were obtained by differential centrifugation, the content of total CE and total PL in mg/g were

determined and their molar ratio was calculated [13]. The isolation of membranes of erythrocytes and mitochondria were carried out by automatic pumping with registration and control on the spectrophotometer „Uvikor D-SH-2089 LKB", „Beckman" company with graphic registration of the peaks of the transition of environments and the measurement of their volumes. The control of the purity of the selection of membranes were performed by using a microscope. Lipid extracts were isolated from 1 ml of erythrocytes and 200 mg of cerebral cortex by method J. Folch et al. [13]. Fractionation of PL was done by the one dimensional ascending method of thin layer chromatography (TLC) [14]. The content of separate phospholipids: phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), lysophosphatidylcholine (LPC), phosphatidylserine (PS), and sphingomyelin (SM) were evaluated by "combustion" of spots with 72 % chloric acid at 200°C until their complete discoloration with the following definition of lipid phosphorus [14]. The content of common PL was calculated based on the sum of individual fractions. As it is known from the scientific literature, the amount of inorganic phosphorus identified in PC, PE, PI, LPC, PS and SM is about 75-80% phosphorus from the remaining phospholipids and phosphate acids (Phosphatidic acid phosphatase (PAP)) [15]. Determination of inorganic phosphorus was carried out by the procedures developed by Chen's modification (2004), which was based on using ascorbic acid as a reducing agent. Measurement of protein in the membrane sample of erythrocytes were performed by using the Lowry method, which combines the biuret reaction and the reaction with Folin's reagent [16].

Statistical analysis of data was conducted by using „Primer Biostatistics 6.0". The criteria of parametric statistics were used. The level of $p < 0.05$ regarded as statistically significant.

Results of the research and discussion

Investigation of damaging effects mechanisms of various pathological factors, including exogenous chemical compounds, on the human and animal body is one of the priority tasks of medicine. Particularly relevant in this case is acquiring the definition of the role of structural and functional state of cell membranes, through which the resistance of the organism to the damaging effect and the restoration of the functioning of organs and systems are substantially mediated, including prescribed pharmacotherapy. The last decade was marked by an active study of the role of membrane mechanisms in the development of a number of diseases, maladaptive changes in the body [17]. It is also known that the implementation of various determinants was largely due to the influence on the phospholipid composition of membranes and the associated activity of marker enzymes. That is, cellular and subcellular membranes, in our case, the membranes of erythrocytes and mitochondria of the brain of rats, in the course of their conformational changes, play a barrier function and they are the first line of defense or targets of primary influence [18]. What is their protective function? A number of researches in our laboratory has proven that cellular or subcellular membranes form the framework of the corresponding structure, separating the contents of the internal environment from the external, thereby maintaining their morphofunctional ability. Maintaining of internal compartment state ensures the stability of the concentration and electrochemical gradients, membrane transport capabilities. On the quantitative and qualitative composition of total lipids, their ratio, the state of phospholipid fractions depends on the stability of the membrane basis, the functional capacity of the multienzyme complexes in the bilayer etc. [19]. On the other hand, it is definitely known that alcohol intoxication as traumatic brain injury discoordinate systemic mechanisms of self-regulation of membranes, which leads to metabolic disorders, acidosis, hypoxia, oxidative stress [20].

Proceeding from the above, the primary task was to study the dynamics of the content of total PL and total CE and their molar ratio in chronic alcohol intoxication and arbitrary recovery. These time intervals are important not only for understanding the period of disease formation, but also for approbation of the possibility, in the future, of medical correction. At the 20th day of alcohol intoxication, the most significant changes develop in the lipid profile of membranes of erythrocytes (table 1).

Table 1. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats with chronic alcohol intoxication and arbitrary recovery (n=7).*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m	4.96±0.16	5.79±0.12	0.86±0.08	4.05±0.21	4.64±0.16	0.87±0.07
		%	100.0	100.0	100.0	100.0	100,0	100.0
2	Alcohol intoxication (20 days)	M±m	6.52±0.20	3.87±0.08	1.68±0.10	7.53±0.27	2.69±0.09	2.80±0.11
		%	131.4*	66.8*	195.3*	186.0*	58.0*	321.8*
3	10 days after alcoholization	M±m	7.56±0.22	3.17±0.09	2.38±0.13	7.01±0.19	3.14±0.11	2.23±0.14
		%	152.4*	54.7*	276.4*	173.1*	67.7*	256.3*
4	20 days after alcoholization	M±m	6.41±0.17	3.91±0.11	1.64±0.09	6.12±0.24	3.56±0.12	1.72±0.10
		%	129.2*	67.5*	190.7*	151.1*	76.7*	197.7*
5	30 days after alcoholization	M±m	5.20±0.10	5.11±0.18	1.02±0.06	5.24±0.15	3.97±0.19	1.32±0.06
		%	104.8*	88.2	118.6*	129.4*	85.6*	151.7*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids

The content of total PL was reduced on a third part (from 5.79±0.12 in the control to 3.87±0.08 mmol/l, P < 0.05), and the content of total CE increased on a third part (from 4.96±0.16 in control to 6.52±0.20 mmol/l, P < 0.05). As a result, the molar ratio of CE/PL changed. It should be noted that in normal condition this ratio is about 1.0. In our study, on the 20th day of the development of alcohol intoxication, the ratio of CE/PL increased almost in 2 times (from 0.86±0.08 to 1.68±0.10, P < 0.05), indicating significant disproportion between total CE and total PL. At the parallel study of the composition of lipids of membranes of the mitochondria of the cerebral cortex, which is the most affected by alcohol intoxication, it was found that on the 20th day of alcohol abuse the content of total PL decreased by 2 times (from 4.64±0.16 in the control to 2.69±0.09 mg/g, P < 0,05), and the content of total CE increased by almost 2 times (from 4.05±0.21 in the control to 7.53±0.27 mg/g, P<0.05), table 1.

Accordingly, the ratio of CE/PL changed more than in 3 times (by 321.8%), indicating that the cerebral mitochondria membranes are more susceptible to the action of alcohol. Observation of the arbitrary restoration of the studied parameters in erythrocyte membranes during 30 days showed that the content of the total CE decreased gradually, on the 10th and 20th days of the study decreased and on 30th days reached the control values (5.20±0.10 and 4.96±0.16 mmol/l in the control, P > 0.05). The content of total PL, similarly to CE, gradually increased and for 30th day probably did not differ from the control (5.11±0.18 and 5.79±0.12 mmol/l in the control, P > 0.05). The ratio of CE/PL was also gradually aligned (0.86±0.08 in the control and 1.02±0.06 on the 30th day of the study). A similar, but less pronounced trend was observed in the membranes of the mitochondria of the cerebral cortex (table 1).

The results of the study indicate that the membranes of the cerebral mitochondria are not only more sensitive to the effects of alcohol, but the restoration of the lipid composition occur less clear and intense, and is more obvious in prolonged terms. At the 30th day of the study neither the content of total CE, nor total PL did not return to ascending values. The evidence of this is the ratio of CE/PL, which for 30 days remained 151.7% higher than in the control (1.32±0.06 at 0.87±0.07 in the control, P < 0 05),

The study of the dynamics of the content of total CE and total PL in the experimental traumatic brain injury in rats demonstrated the same tendency as in chronic alcohol intoxication, but the severity of these changes were much higher (table 2).

Table 2. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats under traumatic brain injury and arbitrary recovery (n=7).*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m	4.32±0.15	5.11±0.11	0.84±0.05	3.98±0.09	4.55±0.11	0.87±0.03
		%	100.0	100.0	100.0	100.0	100.0	100.0
2	1 day after TBI	M±m	6.59±0.17	2.68±0.07	2.46±0.12	7.74±0.22	2.77±0.05	2.79±0.12
		%	152.5*	52.4*	292.8*	194.5*	60.9*	320.7*
3	10 days after TBI	M±m	7.03±0.13	2.85±0.06	2.47±0.11	8.06±0.24	2.65±0.06	3.04±0.13
		%	162.7*	55.8*	294.0*	202.5*	58.2*	349.4*
4	20 days after TBI	M±m	6.28±0.11	3.15±0.09	1.99±0.09	6.43±0.19	2.26±0.09	2.84±0.09
		%	145.4*	61.6*	236.9*	161.5*	49.7*	326.4*
5	30 days after TBI	M±m	6.12±0.10	3.02±0.06	2.02±0.08	5.97±0.16	2.69±0.11	2.22±0.10
		%	141.7*	59.1*	240.5*	150.0*	59.1*	255.2*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids; TBI - traumatic brain injury

One day after the TBI reproduction the content of total PL in erythrocyte membranes decreased almost in 2 times (2.68±0.07 and 5.11±0.11 mmol/l in the control, P < 0,05), and the content of total CE in 1.5 times increased (6.59±0.17 at 4.32±0.15 mmol/l in the control P < 0,05), which led to an increase in the ratio of CE/PL in almost three times. Thus, it was found that TBI leads to marked changes in the content of lipids in erythrocyte membranes, and these changes were so severe that practically 30-days studies in the future did not reveal significant changes in their content. Even in the 30th day of study the content of total PL remained almost 2 times less than in the control, and the content of the total CE remained 1.5 times higher than in the control (table 2). Accordingly, the ratio of CE/PL remained in 2.5 times higher than in the control.

Even more pronounced were the changes in the dynamics of lipid content in the membranes of mitochondria of the cerebral cortex of rats, indicating the severity of brain damage (table 2). One day after the TBI reproduction, the content of total PL decreased by almost in 2 times (2.77±0.05 and 4.55±0.11 mg/g in the control, P < 0.05), and the total CE increased in 2 times (7.44±0.22 and 3.98±0.09 mg/g in control, P < 0.05). Accordingly, the ratio of CE/PL increased by more than in 3 times and reached 320.7 % of the control, P < 0.05. It should be noted that these changes remained until the 30th day of the study, with the appearance of a not significant tendency to normalization, but it was not quite statistically significant.

It is logical to investigate further changes in the content of lipids in combined pathology during reproducing TBI in

rats on the background of alcohol intoxication, which is a complicated and severe pathology [19]. The results indicate that already on the first day of the pathological state changes of the content of lipids in membranes of erythrocytes in rats were more pronounced than in the reproduction of separately alcohol intoxication or TBI (table 3).

Table 3. *Dynamics of total cholesterol content, total phospholipids and their molar ratio in rats under traumatic brain injury on the background of chronic alcohol intoxication and arbitrary recovery (n=7)*

№ n/n	Experiment conditions	Statistical indicators	Erythrocyte membranes (mmol/l)			Mitochondria membranes of the cerebral cortex (mg/g)		
			Total CE	Total PL	Ratio CE/PL	Total CE	Total PL	Ratio CE/PL
1	Control	M±m %	4.64±0.16 100.0	5.45±0.17 100.0	0.85±0.05 100.0	4.15±0.15 100.0	4.40±0.10 100.0	0.94±0.03 100.0
2	Alcohol intoxication (20 days) + TBI (1 day)	M±m %	7.99±0.22 172.2*	1.90±0.10 34.9*	4.20±0.12 494.1*	8.35±0.19 201.2*	1.59±0.05 36.1*	5.25±0.21 558.5*
3	Alcohol intoxication + TBI (10 days)	M±m %	9.01±0.24 194.2*	2.27±0.09 41.6*	3.97±0.13 467.0*	7.79±0.10 187.7*	1.79±0.06 40.7*	4.35±0.19 462.8*
4	Alcohol intoxication + TBI (20 days)	M±m %	8.72±0.19 187.9*	2.21±0.08 40.5*	3.94±0.11 463.5*	7.49±0.12 180.5*	2.11±0.09 47.9*	3.55±0.15 377.6*
5	Alcohol intoxication + TBI (30 days)	M±m %	7.68±0.18 165.5*	3.26±0.10 59.8*	2.35±0.10 276.5*	7.10±0.13 171.1*	2.20±0.10 50.0*	3.23±0.13 343.6*

Note: * - the significant differences between groups as relative to control
CE - cholesterol; PL - phospholipids; TBI - traumatic brain injury

Thus, the content of total PL decreased by almost by three times (to 1.90±0.10 and 5.45±0.7 mmol/l in the control, $P < 0.05$), and the total CE increased by more than in 1.5 times (7.99±0.22 and 4.64±0.16 mmol/l in control, $P < 0.05$). Their ratio was changed catastrophically from 0.85±0.05 to 4.20±0.12, by 5 times ($P < 0.05$). On the 30th day of observation the content of total PL increased almost by 2 times, but did not reach the control values (59.8%, $P < 0.05$), and the content of total CE almost did not change compared to the first day observation (7.99±0.22 at 7.68±0.18 mmol/l in the control, $P < 0.05$). As a result, the ratio remained high and discoordination remained stable and high. Changes that are even more striking were observed in membranes of mitochondria of the cerebral cortex of rats. On the first day of the study total PL decreased by almost three times, and total CE increased twice. Discoordination or ratio of CE/PL reached 558.8% compared with control, $P < 0.05$ (table 3). By the end of the observation period on 30th day the content of these lipids were somewhat aligned, but were far from reference values. The obtained results indicate that the combined pathology, which is TBI on the background of alcohol intoxication, leads to serious consequences, which are the violation of the content of lipids in membranes.

The following results were obtained and there was a logical question, how did combined pathology change the spectrum of isolated phospholipids? Further study of the phospholipid spectrum of erythrocyte membranes in rats under traumatic brain injury on the background of alcohol intoxication showed that this combined pathology not only significantly changed the content of total PL, but also substantially redistributes the content of their individual fractions. To date, the boundaries of the content of individual PL fractions have been thoroughly studied. However, their role is very diverse not only with various functional symptoms of the body, but also with various pathological manifestations. Unfortunately, these data are quite controversial, ambiguous and to make qualitative generalizations is difficult for them [21]. On this basis, we focus on the main properties of PL. It is known, that PL contains alcohol, glycerol or sphingosine, free fatty acids and phosphoric acid [15]. Some of them contain nitrogen-containing compounds such as choline, ethanolamine, inositol, serine – PC, PE, PI, PS [15]. All PL are divided into 2 groups. I group form glycerophospholipids (PC, PE, PI, PS), the main function of which is the formation of lipid bilayer membranes, regulation of the activity of membrane enzymes through the maintenance of the stability, viscosity and permeability of membranes. Their important function is also to provide a calcium-dependent mechanism for the transfer of hormonal signal into the cells. This group also includes LPC, which as a result of hydrolysis by phospholipase A_2 forms prostaglandins and leukotrienes [15]. The latter one form the immune-biological properties of the organism. II group – sphingophospholipids, which include SM, which causes two main functions in the body – regulation of the transmission of the nerve impulse and internal homeostasis. In addition, the PL can be divided into light oxidized, which include PC and PE and hardly oxidized (lysoforms), to which in the first place refer LPC, SM [22].

Investigation of the dynamics of the content of phospholipid fractions in this work we conducted on membranes of erythrocytes of rats with combined pathology, taking into account the greater availability of erythrocytes

and laborious method of determining individual fractions of PL (table 4). Studies have shown that for the quantitative content in the control of individual PL membranes of erythrocytes are located as follows: 35.9% occupied by PC; 20.4% - PE; 18.0% - SM; 8.6% - PI; 7.2% - LPC; 4.5 % - PS and 5.4 % - PAP. Thus, we can conclude that the number in the PL most commonly are PC, PE and SM. Their share accounts for 74.3%: it can be assumed that in the functional plan their participation in regulation of viscosity, permeability of membranes (PC, PE) and receptors ability (SM) support is more important. One day after the TBI reproduction on the background of alcohol intoxication, almost twice times pronounced decreasing in the content of total PL, a sharp discoordination of various PL fractions was observed. The number of PC and PE were decreased by almost by 3.5 times, and PI and PS were decreased almost by 2 times. At the same time, the number of LPC were increased almost by 2 times. Increasing the content of SM was statistically significant, but was only 117.5 % (table 4). These data are objective evidence of the above assumption. It is also necessary to emphasize the fact that more than a threefold decrease was attributed to PC and PE, which belong to the class of light oxidized PL, and PE and PS, in addition, contain polyunsaturated fatty acids. The content of hardly oxidized PL, such as LPC and SM, on the contrary increased. These data reflect the fact that the increase in LPC content indicates the activation of phospholipase A_2 and, consequently, an increase in the number of prostaglandins and prostacyclin formation [15]. This fact confirmed the discoordination of immune-biological protection of the body, increasing of catabolic and destructive processes in the erythrocyte membranes.

In subsequent monitoring periods, there was a gradual restoration of the content of total PL and their individual fractions, but at 30th day, it did not reach control values. Despite the significant increase in the level of total PL, on the 30th day of the study after the reproduction of TBI on the background of alcohol intoxication, the level of PC, PE and PI remains significantly lower compared to control, and the content of LPC and SM statistically significant were high. The content of the PS was close to the control values. That is, the discoordination of the metabolism of individual fractions of PL was so pronounced that even on the 30th day of arbitrary recovery did not reach the ascending values, which again emphasizes the seriousness of the morphofunctional state disturbance of membranes of red blood cells.

Conclusions

1. Studies have shown that chronic alcohol intoxication in rats and reproduction of an experimental traumatic brain injury separately causes a significant change in the content of total phospholipids and total cholesterol, and their molar ratio in the membranes of erythrocytes and mitochondria of the cerebral cortex. Particularly pronounced were these changes in the studied objects in combined pathology, that is, at the traumatic brain injury on the background of chronic alcohol intoxication.

2. Discoordination of lipid metabolism was unidirectional and consisted of a significant reduction in the content of total phospholipids and increased total cholesterol, which led to a violation of the molar ratio of CE/PL in the direction of increasing the coefficient, which is normally is about 1.0.

3. The results of the work show that the arbitrary restoration of the studied parameters during chronic alcohol intoxication on 30th day of observation significantly approached the control values, and when reproduced TBI, where the violations were more pronounced, on 30th day did not return to the ascending values. Under combined traumatic brain injury on the background of chronic alcoholic intoxication on 30th day the shift in lipid content only gained a marked tendency to normalize. That is, the discoordination of the lipid content was quite pronounced.

4. Along with the changes in the content of total PL, total CE and their molar ratio under combined TBI on the background of chronic alcohol intoxication in the membranes of erythrocytes, there was a significant shift of the spectrum of individual fractions of phospholipids. After 1 day of reproduction, the content of PC and PE decreased in 3.5 times, and in 2 times the PI and PS, at the same time, the content of LPC increased by almost 2 times.

5. A threefold decrease in the content of PC and PE, light oxidized PL, indicates a violation of the formation of lipid bilayer of membranes of erythrocytes and the activity of embedded in them enzyme systems due to changes in viscosity and penetration of membranes.

6. In subsequent monitoring periods there was a gradual restoration of individual fractions of PL, but on the 30th day of the study, it did not reach the control values. That is, the discoordination of the metabolism of individual fractions of PL was so pronounced that even on 30th day of arbitrary reproduction did not reach the ascending values, which again emphasizes the seriousness of the morphofunctional state disturbance of membranes of erythrocyte membranes.

Prospects for further research.

An important task for the future research should be the further study of the role of phospholipids in the formation of the effects of traumatic brain injury on the background of chronic alcohol intoxication and development of the methods of pharmacological correction of targeted action.

Declaration of conflict of interests

Authors certify the absence of conflict of interests.

Authors' contributions

All authors contributed equally to the research, data analysis, and writing of the manuscript. Final manuscript was read and approved by all authors.

Table 4. *Dynamics of content of phospholipid fractions in erythrocyte membranes of rats under traumatic brain injury on the background of chronic alcoholic intoxication and arbitrary recovery (mg / 100 ml of erythrocytes, n = 7).*

№ n/n	Experiment conditions	Statistical indicators	Total PL	Fractions of PL (%)						
				Phosphatidylcholine (PC)	Phosphatidylethanolamine (PE)	Phosphatidylinositol (PI)	Lysophosphatidylcholine (LPC)		Sphingomyelin (SM)	Phosphatidic acid phosphatase (PAP)
1	Control	M±m % (%TFPL)	275.5±4.1 100.0 100.0	98.9±3.1 100.0 35.9	56.2±2.4 100.0 20.4	23.7±1.0 100.0 8.6	19.8±1.4 100.0 7.2	12.3±0.9 100.0 4.5	49.6±1.4 100.0 18.0	15.0±0.9 100.0 5.4
2	Alcohol intoxication (20 days) + TBI (1 day)	M±m % (2-1) (%TFPL)	164.8±1.8 59.8* 100.0	28.8±1.8 29.1* 17.5	16.1±1.1 28.6* 9.8	12.7±0.8 53.6* 7.7	38.6±1.5 194.9* 23.4	7.4±0.8 60.2* 4.5	58.3±1.5 117.5* 35.4	2.9±0.2 19.3* 1.7
3	Alcohol intoxication + TBI (10 days)	M±m % (3-1) (%TFPL)	175.9±1.9 63.8* 100.0	29.0±1.7 29.3* 16.5	15.9±0.9 28.3* 9.0	14.1±0.9 59.5* 8.0	39.7±1.3 200.5* 22.6	8.5±0.7 69.1* 4.8	61.7±1.7 124.5* 35.1	7.0±0.4 46.7* 4.0
4	Alcohol intoxication + TBI (20 days)	M±m % (4-1) (%TFPL)	193.8±2.1 70.3* 100.0	35.2±1.8 35.6* 18.2	16.7±0.8 29.7* 8.6	13.9±0.7 58.6* 7.2	45.8±1.5 231.3* 23.6	9.7±0.9 78.9* 5.0	66.3±1.6 133.7* 34.2	6.2±0.3 41.3 3.2
5	Alcohol intoxication + TBI (30 days)	M±m % (5-1) (%TFPL)	214.5±2.8 77.8* 100.0	51.9±2.2 52.5* 24.2	40.3±1.4 71.7* 18.8	12.8±0.6 54.0* 6.0	31.2±1.2 157.6* 14.5	10.5±0.6 85.4* 4.9	59.7±1.5 120.4* 27.8	8.1±0.4 54.0* 3.8

Note: * - The difference between two groups (such as an experiment vs. control group) is judged to be statistically significant when p = 0.05 or less.
% - total fraction of phospholipids (TFPL) - fraction percentage relative to total PL

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RESEARCH ARTICLE

Importance of urodynamic testing prior to treatment for overactive bladder in women

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What is not known yet about the topic

The use of urodynamics in the diagnosis of overactive bladder remains controversial.

Research hypothesis

Urodynamics appears to influence treatment decisions made by clinicians and patients in determining management pathways in women presenting with overactive bladder.

Article's added novelty on this scientific topic

Women treated based on urodynamic investigation appear to have greater reductions in symptoms than those who do not. Urodynamic investigation is mandatory for diagnosis and treatment among women with symptoms of overactive bladder; determining the diagnosis only on the basis of urinary symptoms would lead to underdiagnosis of detrusor overactivity.

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Abstract

Introduction. Overactive bladder (OAB) is a common and chronic complex of symptoms that increases in prevalence with advancing age and has a known adverse effect on the quality of life. OAB is a highly prevalent condition affecting 16.6% people from Europe. Women are more commonly affected, and there is an increased incidence with age. Studies in the United States suggest a prevalence of up to 43% in women. The use of urodynamics in the diagnosis of OAB remains controversial. Although it is a gold standard diagnostic test for detrusor overactivity, it is an invasive procedure and therefore should be limited to those with refractory OAB.

Material and methods. A prospective and randomized study was performed in 60 patients with OAB symptoms who followed behavioral therapy without any effect. The study cohort was divided in two groups. 30 patients (group A) with the mean age of 40 years were treated without a prior urodynamic study, and 30 patients (group B) with a mean age of 41.5 years with overactive detrusor, underwent a urodynamic testing prior to pharmacotherapy based on EAU guidelines that recognize the benefit from addition of Mirabegron 50 mg/day to Solifenacin 5 mg/day, and on the AUA guidelines that recommends combination therapy in patients with OAB. The study was performed during 2019-2022, at the Department of Urology and Surgical Nephrology, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.

Results. The success rate (61%) in the group A of patients was lower than in group B (81%). The proportion of patients who had urge urinary incontinence (UUI) (OAB wet) rather than frequency-urgency (OAB dry) in this series was high (50%), and this may have been a significant factor in our success rate. According to the results of the questionnaire, the clinical manifestations have improved after treatment, however in 50% of cases of urinary frequency and in 20% of urinary urgency remained unchanged. The symptomatology and urodynamic did not display different behavior between the groups. The mean post-treatment score for group A was 11.7±3.27 and for group B was 15.32±2.14. Ten subjects (8 receiving pharmacotherapy from group A and 2 from group B) presented with adverse events. The most frequent reported adverse events were dry mouth (15%), dyspepsia (6%), and headache (9%). Other than dry mouth, no adverse event occurred in >10% of subjects.

Conclusions. Urodynamics can influence the treatment decisions in determining treatment pathways in women presenting with OAB. Women treated based on UDS diagnoses appear to have greater reductions in symptoms than those who do not.

Keywords: overactive bladder, urodynamic, pharmacotherapy, lower urinary tract symptoms.

Introduction

Overactive bladder (OAB) is a common and chronic symptom complex that increases in prevalence with advancing age and has a known adverse effect on quality of life. OAB is a highly prevalent condition affecting 16.6% of the European population. Women are more commonly affected, and there is an increased incidence with age. Studies in the United States suggest a prevalence of up to 43% in women and 27% in men older than 40 years of age. There are significant differences in racial/ethnic groups with OAB being highest in African Americans. The International Continence Society (ICS) has recently defined OAB as urgency with or without urgency urinary incontinence (UUI), usually with frequency and nocturia. This definition is based on symptoms and does not require urodynamic investigation (UDS). It is important to note that many clinicians use urodynamics to diagnose detrusor overactivity (DO) before initiating treatment. OAB is often defined clinically by urodynamic variables thought to be responsible for the symptoms [1, 2].

A focused history is paramount in diagnosing OAB. It is critical to assess onset of symptoms as well as aggravating and alleviating factors and 24/h pad use. Physical examination should include the assessment of the genitourinary system, as well as digital rectal and prostate examination and in men and vaginal examination in women. Urinalysis, by dipstick initially, should be performed to rule out hematuria and infection. Validated questionnaires are available to assess effects on quality of life as well as symptoms. Bladder diaries or frequency-volume charts provide an accurate and reliable measure of voiding patterns. Imaging of the urinary tract is not required for diagnosis but may be used as saddditional test in those patients with suspected bladder outflow obstruction [2, 3].

The use of urodynamics in the diagnosis of OAB remains controversial. Although the gold standard diagnostic test for detrusor overactivity, it is an invasive procedure and therefore should be limited to those with refractory OAB. The National Institute for Health and Care Excellence (NICE) advises urodynamics prior to third-line therapy, European Urological Association (EAU) only if findings may change management and the American Urological Association (AUA) for patients with complicated OAB (such as those with concurrent urethral dysfunction or in those in whom the diagnosis is not clear [2, 4].

In the UK, a nationally funded National Institute for Health Research superiority trial has just finished recruitment looking at the usefulness of urodynamics prior to treatment for refractory OAB syndrome [2].

The consensus is that UDS is not indicated in patients with OAB prior to conservative or medical therapy. The main area of debate is that many clinicians believe that UDS is indicated in refractory OAB and only when initial therapy fails and it should be performed prior to any surgical intervention including minimally invasive procedures, such as sacral neuromodulation or onabotulinum toxin type A injection. They support this opinion with studies that showed that UDS is an invasive expensive tool, time consuming, and does not influence the initial management strategies [5].

Combination therapy (antimuscarinic and beta3-agonist) may be considered in patients refractory to monotherapy. Co-administration appears to improve efficacy with minimal increase in the side effect profile. Solifenacin and Mirabegron combination therapy (in doses of 5mg and 25mg or 5mg and 50mg, respectively) is reported to have a statistically significant decrease in number of incontinence episodes and micturition compared with Solifenacin or Mirabegron alone [2, 4, 6].

The aim of the present study was to analyze whether patients with OAB need different treatment management and if it is dependent on establishing on urodynamics study presence of overactive detrusor contractions.

Material and methods

A prospective and randomized study was performed in 60 patients with OAB symptoms who followed behavioral therapy without any effect. The study cohort was divided in two groups. 30 patients (group A) with the mean age of 40 years were treated without a prior urodynamic study, and 30 patients (group B) with a mean age of 41.5 years with overactive detrusor, underwent a urodynamic testing prior to pharmacotherapy based on EAU guidelines that recognize the benefit from addition of Mirabegron 50 mg/day to Solifenacin 5 mg/day, and on the AUA guidelines that recommends combination therapy in patients with OAB. The study was performed during 2019-2022, at the Department of Urology and Surgical Nephrology, *Nicolae Testemitanu* State University of Medicine and Pharmacy, Chisinau, Republic of Moldova.

In this study, fundamental ethical principles of research have been respected. All patients gave informed consent before study entry. The study protocol was endorsed positively by *Nicolae Testemitanu* University Research Ethics Committee (Minutes No. 24, 05.03.2021). Patients who underwent the surgical procedure, were asked to explain that they understood the nature of the surgical procedure and after they gave the agreement for operation by signing the informed consent.

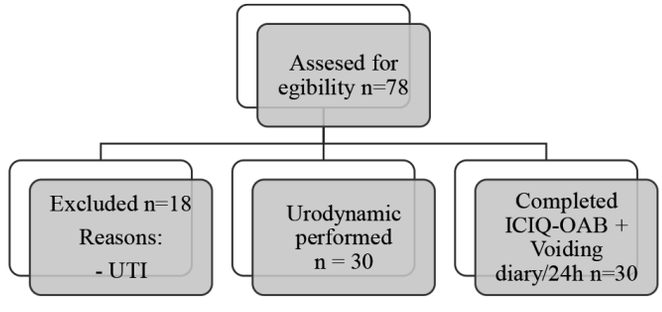

Fig. 1. Flowchart of prospective study design

All patients had moderate and severe clinical manifestations according to ICIQ-OAB. At 3-month follow-up, patients in both groups underwent the ICIQ-OAB questionnaire and voiding diary/24h.

The urodynamics were carried out in accordance with the trust standard operating procedures, which were based on the good urodynamic practice. Uroflowmetry was performed with women voiding in a private room on a flowmeter. Filling cystometry was performed in sitting position at 100 mLs/min rate, followed by provocation maneuver and ended with voiding cystometry. The clinician who performed the UDS recorded the findings and diagnosis on the UDS report and inside the patient's notes.

Urodynamic studies were commonly performed for the diagnosis of OAB and DO using urodynamic equipment Medica S.P.A. Memphis Division (Medolla-Italy). Women with infravesical obstruction, detrusor underactivity and detrusor overactivity with inadequate contractility were excluded from this study.

Urodynamic parameters were PVR from ultrasound, maximum cystometric capacity (MCC), maximum detrusor pressure (MDP), maximum urinary flow pressure (PdetQmax), maximum urinary flow rate (Qmax) and bladder compliance (BC). BC was calculated using the ratio of urine volume to detrusor pressure, being considered low when $\Delta V/\Delta Pdet$ was ≤ 30-40 ml/cmH_2O, despite unexplained and insufficient data on the presence of normal values.

The actual procedure was explained to all patients in a clear manner by providing a scenario, instructions on how to report the 4 sensations during the cystometry. Patients signed the informed consent before the procedure.

Rectal urodynamic catheter was inserted ~10 cm depth, after what the urethra was catheterized using a 7Fr double lumen urodynamic catheter. The bladder was emptied after confirmation of the lack of residual urine based on the urodynamic investigation. Patients placed in a sitting position after the filler wires have been connected. The transducers were placed at reference heights according to ICS standards with the respective calibration of atmospheric pressures. The working of transducers was confirmed after patients' cough and the filling of the bladder was performed with saline solution prepared at room temperature (filling speed 20 ml/min). The filling was stopped once the patient reached the maximum cystometric capacity, then the patient urinated.

Statistical data analysis was performed using unifactorial dispersion analysis designed in Microsoft Excel 2019 database and IBM SPSS Statistics 22 software, using the standard and paired t-tests, with a significance level of 0.05. The categorical data were presented as absolute and relative values and the continuous data – in the form of mean and standard error, or as a percentage of results, comparing results before and after procedure.

Results

Urodynamics appears to influence treatment decisions made by clinicians and patients in determining treatment algorithms in women presenting with OAB. Women with DO were three times more likely than women with normal diagnosis to have been prescribed bladder relaxants. This could be interpreted as those who were shown to have DO either received prescribed bladder relaxant tablets more or patient compliance with taking the treatment was better. Women with a diagnosis of DO were 15 times more likely to have onabotulinum toxin type A injection than no treatment, which may at least partly explain the improved ICIQ-OAB scores in this group compared with group A.

Factors identified in this analyses that may influence the result of therapy include age, previous continence surgery, previous use of medication for bladder symptoms, menopausal status and parity. Studies in the past have shown that pelvic floor symptoms have a different impact on women of different ages. Progesterone and estrogen may exert or influence the female nerves, as well as influencing bladder contractions and voiding frequency.

Table 1. *Voiding diary parameters in patients with overactive bladder symptoms.*

Voiding diary parameters	Group A		Group B	
	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)
TVV / 24h (ml)	1314±645	1565±168	1280±635	1679±168
FBC (ml)	163.1±123.9	338±69	157.1±115.5	378±76
IN	2.86	0.7±0.1	2.75	0.5±0.1
IPN (%)	28.7±9.4	15.8±5.1	27.6±7.4	11.8±3.1
DV	11.3±1.68	5.1±2	14.1±1.45	6.1±2
TUFS	31.7±7.8	7.7±3.8	35.2±6.2	5.7±3.2

Note: TVV – total voided volume; FBC – functional bladder capacity; IN – Index of nocutia; IPN – Index of nocturia polyuria; DV – daytime voiding; TUFS – total urgency and frequency score.

Based on voiding diary, before and after treatment, there were analyzed indices of total voided volume, functional bladder capacity, nocturia index and nocturia polyuria index, number of daytime voiding and total index of urgency and frequency urination.

After 3 months of treatment, in both groups, patients obtained normal ranges of indices, and this correlates with the disappearance of clinical manifestation. We have observed what bladder voiding diary results were with respect to urgency, total voided volume, average voided volumes, and maximum functional capacity in relation to clinical and urodynamic diagnoses.

Table 2. *ICIQ-OAB values in patients with overactive bladder symptoms.*

ICIQ-OAB Domain	Group A		Group B	
	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)
Urinary frequency	100%	75%	100%	50%
Urinary urgency	100%	75%	100%	20%
Nocturia	100%	50%	100%	0%
Urge urinary incontinence	100%	25%	100%	0%
OAB-QoL	Severe	Mild	Severe	Light

Note: ICIQ-OAB – International Consultation on Incontinence Questionnaire Overactive Bladder Module; OAB-QoL – International Consultation on Incontinence Questionnaire Overactive Bladder Quality of Life Module.

All validated self-report questionnaires quantifying OAB symptoms (daytime urinary frequency, nocturia, urinary urgency and urge urinary incontinence) and quality of life were completed by all women prior and after conservative treatment. A significant decrease of symptoms in patients from group B and of negative impact of LUTS/OAB on daily indoor and outdoor activity, physical and social activity was reported by patients following the urodynamic tests.

The ICIQ-OAB questionnaire indices and their improvements after pharmacotherapy in group B are shown in table 3. According to the results of the questionnaire, the clinical manifestations have improved after treatment, however in 50% of cases of urinary frequency and in 20% of urinary urgency remained unchanged.

Table 3. *The effect of pharmacotherapy in patients with overactive bladder symptoms.*

	Group A	Group B
Success	61%	81%
Failure	39%	19%

The success rate (61%) in the group A of patients was lower than in group B (81%). The proportion of patients who had urge urinary incontinence (OAB wet) rather than frequency-urgency (OAB dry) in this series was high (50%), and this may have had a significant impact on our success rate.

Table 4. *The effect of pharmacotherapy in patients with overactive bladder symptoms compared by ICIQ-OAB questionnaire.*

	Group A		Group B	
	ICIQ-OAB		ICIQ-OAB	
	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)	Pre-treatment (n = 30)	Post-treatment (after 3 months) (n = 30)
Solifenacin + Mirabegron	Severe (100%)	Severe (25%) Mild (75%)	Severe (100%)	Severe (19%) Mild (70%) Absence of symptoms (11%)

Note: ICIQ-OAB – International Consultation on Incontinence Questionnaire Overactive Bladder Module.

Patients from group A did not continue the conservative treatment for a long period due to lack of results and persistence of OAB symptoms after 2-3 weeks of treatment, but patients from group B having a confirmation of diagnosis of OAB with overactive detrusor were more confident in their treatment ant continued for longer period administration of pharmacotherapy.

The behavioral therapy was combined with pharmacotherapy in both groups for obtaining better results after 3 months of conservative treatment.

Both groups improved well during antimuscarinic treatment associated with Mirabegron, the severe manifestation of symptoms from baseline in urinary frequency or UUI episodes disappearing after 3 months.

Table 5. *Urodynamic parameters in whom pharmacotherapy was successful and in whom it failed.*

		Success	Failure
	Female	16 (81%)	4 (19%)
Urodynamic parameters			
Uroflowmetry	Maximum voided volume (ml)	132.7±136.7	95.2±83.8
	Qmax (ml/s)	9.8±4.1	8.4±6.1
	Qave (ml/s)	2.2±1.6	2.1±1.9
Cystometry	FS (ml)	79.8±56.3	65.5±45.7
	FDV (ml)	117.8±103.2	101.8±100.2
	SDV (ml)	162±125	134±109
	MCC (ml)	183.4±139.8	141.1±138.2
	MDP (cmH_2O)	45.9±23.9	32.6±21.9
	Number of contractions	3.9±1.1	5.6±2.1
BC (ml/cm H_2O)		10.6±11.5	11.7±12.8
CI		124.6±39.4	133.4±36.3
PVR (ml)		4.9 (0 - 10)	2.4 (0 - 10)

Note: Qmax – maximum flow rate; Qave – average flow rate; FS – first sensation of bladder filling; FDV – first desire to void; SDV – strong desire to void; MCC – maximum cytometric bladder capacity; MDP – maximum detrusor pressure; BC – bladder compliance; CI – Index of detrusor contractility; PVR – post-void residual urine volume.

Standard filling phase urodynamic parameters did not predict a successful response to pharmacotherapy although there is suggestion that sensory data is important. In patients from group B that failed the treatment, the urodynamic values were lower and the drug was less efficient than in subjects that had higher urodynamic values.

The symptomatology and urodynamic data did not display different values between the groups. The mean

post-treatment score for group A was 11.7±3.27 and for group B was 15.32±2.14.

Ten subjects (eight receiving pharmacotherapy from group A and two from group B) presented adverse events. The most frequent adverse events reported were dry mouth (15%), dyspepsia (6%), and headache (9%). Other than dry mouth, no adverse event occurred in >10% of subjects.

Discussion

There are many studies that have utilized questionnaires and voiding diary for treatment response and success, however, this is a study that evaluates the importance of accuracy of urodynamic values in predicting the good result of treatment. The results of this study demonstrate that the UDS has an objective ability to establish a diagnosis of DO and predict a better result after pharmacotherapy.

The study was prospective, recruited consecutive women presenting with lower urinary tract symptoms and achieved its target sample size. The prevalence of DO in our study was in all women from group B. Ambulatory UDS was offered to 100% of women who presented OAB symptoms, all the criteria for a high-quality test accuracy evaluation have been met. Women were only included in group B if they had accepted the disclaimer at the end of the completing the ICIQ-OAB questionnaire.

The usefulness of UDS has been challenged in clinical practice. Some may question the relevance of DO when there are many women who have DO without any urgency or incontinence. The diagnosis of DO does not alter treatment outcomes for interventions like antimuscarinics, but the results from our study reveal the success rate of treatment.

According to the latest recommendations of the ICS and International Consultation for Incontinence (ICI) in 2016, UDS have an overall accepted indication to assess LUTS function and LUTD, especially when it may have a therapeutic consequence, and may change the therapeutic options or when it is performed as part of lower urinary tract assessment or research. It is considered the "gold standard" functional test to assess LUTS [5].

Cho *et al.* (2015) investigated the role of UDS in female patients with OAB. Clinical and urodynamic data of 163 women with OAB were analyzed. They concluded that OAB symptoms were not useful for predicting presence of voiding dysfunction and for this UDS may be necessary for accurate diagnosis in women with OAB symptoms [7, 8].

Many researchers believe that UDS is indicated only in patients with OAB symptoms after failure of first-line therapy. They believe that UDS will not change the initial management strategies in such patients in addition to its cost and invasiveness [5].

Conversely, many others believe that UDS is still mandatory in female patients with OAB, as treatment based on symptoms alone may lead to under diagnosis of DO and storage symptoms that can be detected by UDS which will ultimately alter the diagnosis and management plan. One retrospective single-center study confirmed that there is no association between subjective symptoms severity in patients with OAB and objective measures. This confirmed the role of UDS as an objective measure that is needed for better assessment [5, 8, 9].

When considering pretreatment UDS prior to third-line therapies, there are several questions to consider: (1) Does UDS predict treatment outcomes? (2) Do treatments have an impact on UDS findings? (3) If there is an impact, does it matter? Urodynamics should be used judiciously in such patients. However, for patients with known or suspected voiding phase dysfunction, those with rapidly changing symptoms, those in whom the diagnosis is not clear, those who have medical or urological histories that can affect outcomes of treatment, and in those with known or suspect neurological disease, urodynamics is often indicated, useful, and in some cases essential [8, 10, 11].

The role of UDS continues to be a heavily debated subject for assessing female patients with LUTS. Nonetheless, UDS remains a valuable diagnostic test that provides vital information, to both the surgeon and the patient prior to invasive treatment, with minimal morbidity.

Mirabegron is a beta-agonist that acts to facilitate bladder detrusor relaxation. Mirabegron has demonstrated sustained improvements in number of micturition and incontinence. Intolerable side effects, such as dry mouth, are statistically less compared with antimuscarinic therapy. In addition, although there are concerns regarding blood pressure rises, this remains small and Mirabegron is efficacious and safe, with no difference in treatment-emergent hypertension compared with placebo. The Medicine and Healthcare products Regulatory Agency recommends the use of Mirabegron with caution in those patients with stage 2 hypertension (systolic blood pressure ≥160 mmHg and/or diastolic ≥100 mmHg). It is contraindicated in patients with severe uncontrolled hypertension (systolic blood pressure ≥180 mmHg and/or diastolic ≥100 mmHg) [2, 9, 12, 13].

Combination therapy with 10 mg Solifenacin greatly increased its side-effect profile with only marginal benefits in efficacy. Although EAU guidelines recognize there may be more benefit from addition of Mirabegron to Solifenacin 5 mg, rather than increasing Solifenacin to 10mg, currently only the AUA recommends combination therapy in patients who are refractory to either in the treatment algorithm [6, 14, 15].

Subsequent treatment was found to be highly associated with diagnosis in both groups and had a good improving of symptoms and quality of life.

Conclusions

Urodynamics may influence the treatment decisions in determining treatment pathways in women presenting with OAB. Women treated based on UDS diagnoses appear to have greater reductions in symptoms than those who did not.

Urodynamics should be used especially for patients with known or suspected voiding phase dysfunction, those with rapidly changing symptoms, those in whom the diagnosis is not clear, those who have medical or urological histories

that can affect outcomes of treatment, and in those with known or suspected neurological disease.

Abbreviations

AUA – American Urological Association; BC – bladder compliance; DO – detrusor overactivity; EAU – European Urological Association; ICI – International Consultation for Incontinence; ICIQ–OAB – International Consultation on Incontinence Questionnaire Overactive Bladder Module; ICS – International Continence Society; LUTD – lower urinary tract dysfunction; LUTS – lower urinary tract symptoms; MCC – maximum cystometric capacity; MDP – maximum detrusor pressure; NICE – National Institute for Health and Care Excellence; OAB – Overactive bladder; PdetQmax – maximum urinary flow pressure; PVR – post–void residual urine volume; Qmax – maximum urinary flow rate; UDS – urodynamic investigation; UK – United Kingdom; UUI – urgency urinary incontinence.

Declaration of conflicting interests

The author declares the absence of any conflict of interest in the elaboration of this article.

Authors' contribution

Both authors contributed equally to the development of the manuscript and approved its final version.

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RESEARCH ARTICLE

Six years' experience of participation of the Institute of Emergency Medicine from Chisinau, Republic of Moldova in the Registry of Stroke Care Quality

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What is not yet known on the issue addressed in the submitted manuscript

The quality level of the management of stroke patients, considering that cerebrovascular events are the second cause of mortality in our country.

The research hypothesis

The level of medical care provided to stroke patients was affected by the COVID-19 pandemics.

The novelty added by manuscript to the already published scientific literature

The detected gaps in stroke management and the possibility of correcting them.

Abstract

Background. The Institute of Emergency Medicine from Chisinau has joined the Registry of Stroke Care Quality (RES-Q) in 2017.

Material and Methods. We have analyzed the stroke patients' data introduced in RES-Q between 20172022. Baseline characteristics, stroke-related factors, processes of care, and discharge destination were examined.

Results. Data were available for 489 stroke patients. The proportion of patients with ischemic strokes receiving thrombolysis therapy has substantially increased from 2.78% in 2017 to 20% in 2022, and the median door-to-needle time decreased from 85 minutes (2017) to 48 minutes (2021). Thrombectomy began to be performed in 2018 at the Institute of Emergency Medicine, the rate of stroke patients receiving thrombectomy increased from 3.23% in 2019 to 10% in 2022, median door-to-groin time decreased from 228.5 minutes in 2019 to 102 minutes in 2021. More than 80% of patients received secondary prevention therapies that were level-1 evidence-based processes of care: antihypertensive agents (89.47% – 98.44%), antiplatelet drugs (81.63% – 100%), anticoagulants for patients with atrial fibrillation (25% in 2018 vs 100% in 2021), but cholesterol-lowering medication was prescribed to approximately a half of the participants (44.23% – 60.0%). There were more deaths in the pandemic years (36.67% in 2021 vs 18.99% in 2017).

Conclusion. Most hospitalized stroke patients admitted to the Institute of Emergency Medicine received evidence-based care. The COVID-19 pandemic has left its mark on the number of stroke hospitalizations, but the quality of in-hospital stroke care was not dramatically affected.

Keywords: stroke, RES-Q registry, stroke management.

Introduction

Cerebrovascular pathology is one of the main causes of morbidity, mortality, and disability, both at a global and national level in the Republic of Moldova. In the Republic of Moldova, there were 77748 patients with cerebrovascular pathologies in 2020 vs. 79691 in 2019, according to the National Public Health Agency data published, with cerebrovascular pathologies having a prevalence of 270.7 in 2020 vs. 277.4 in 2019 per 10,000 people. The incidence of cerebrovascular pathologies was 5523 in 2020 and 7320 in 2019, which represents 19.2 in 2020 vs. 25.5 in 2019 per 10,000 people.

Clinical registries play an important role in the health services assessment and, indirectly, determine their improvement. Since 2016, Moldova is part of the ESO-EAST project

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(European Stroke Organization Enhancing and Accelerating Stroke Treatment) of the European Stroke Organization [1-5]. ESO EAST aims to improve the quality of stroke care in the population of Eastern European countries, which is delivered from the admission of a patient through his or her discharge from that hospital, as in Eastern European countries the incidence and prevalence of this disease are impressive and constantly progressing. Continuous monitoring of stroke care quality allows hospital-to-hospital and country-to-country benchmarking and identification of the gaps and needs in health care. The quality of stroke care relates to imaging, acute care, and prevention of complications, secondary prevention, and rehabilitation. Therefore, the main objective of the Registry of Stroke Care Quality (RES-Q) project is to improve the quality of healthcare provided to stroke patients by translating the data collected by RES-Q into effective health policies at the national and international level by collecting data over a period of one month or several years consecutively [5-8].

The Institute of Emergency Medicine from Chisinau joined the registry in 2017 and has enrolled 489 patients.

Our aim was to investigate whether in-hospital quality indicators in stroke patients changed between 2017 – 2022.

Materials and methods

A retrospective analysis of all consecutive acute stroke patients from the Institute of Emergency Medicine of Chisinau, Republic of Moldova, was performed. We have analyzed the data introduced in the RES-Q registry between March 1 and March 31, 2022, and compared it with the same period for 2017, 2018, 2019, 2020, and 2021.

A wide range of factors were recorded in the RES-Q registry, including patient characteristics and care received in hospital. The factors investigated in the study included: sociodemographic, such as age and sex; health behaviors and comorbidities, such as presence of atrial fibrillation (AF) and smoking status; stroke-related factors, such as stroke severity assessed by the National Institutes of Health Stroke Scale (NIHSS); stroke type; in-hospital stroke; time from stroke onset to hospital arrival, and door-to-needle time for intravenous thrombolysis; discharge information, such as length of hospital stay and discharge destination; and evidence-based processes of care provided while in hospital.

The data collection in our country is performed as part of routine clinical practice and quality assurance and therefore does not require special approval from local ethical committees. The protocol of this study is approved by the Ethical Committee of St. Anne's University Hospital, Czech Republic.

Results

The Institute of Emergency Medicine from Chisinau joined the registry in 2017 and has enrolled 489 patients. The COVID-19 pandemic has left its mark on the number of stroke hospitalizations, a fact observed in all countries of the world, including the Republic of Moldova, as evidenced by the data of the RES-Q registry (tab. 1) [9-13].

Patient Characteristics

In 2021, there were more hospitalized men (58.3% in 2021 vs. 48.65% in 2018), more patients with ischemic stroke (86.67% in 2021 vs. 60.78% in 2019). No age (68 years) difference was noted (tab. 1). More than one fourth of the patients (tab. 1) were current smokers, and the proportion was four times greater in men compared to women.

The presence of atrial fibrillation (patients known to have AF) for ischemic stroke and TIA was found in 35.5% of patients in 2022, compared to 50% in 2020 (tab. 1). AF was detected during hospitalization for ischemic stroke and TIA in 6.5% of patients in 2022 vs. 5.77% in 2020 by electrocardiogram (ECG).

Stroke Severity and Stroke Type

At admission, the median National Institute of Health Stroke Scale score indicating stroke severity was between 7 in 2017 and 12 points in 2021 Tab. 1).

There were 21.25% – 35.9% of cases presenting with a loss of consciousness (drowsy or comatose) at admission. The majority of patients were found to have ischemic stroke (60.78% – 91.14%), followed by intracerebral hemorrhage (7.6 – 13.33%), while only a very small proportion had subarachnoid hemorrhage (1.27% – 2.1%).

Table 1. *Characteristic of RES-Q Registrants for Stroke in the Institute of Emergency Medicine for one month (March), 5 years consecutively (2017 – 2022).*

Year	**2017**	**2018**	**2019**	**2020**	**2021**	2022
Number of cases	79	111	102	60	60	71
Sociodemographic						
Age in years, median	69	69	68	69	66	67
Prestroke health						
Current smoker (%)	20.31	23.28	12.64	10.86	20.2	18.3
The presence of AF (known AF) for ischemic stroke and TIA (%)	36.11	26.96	23.46	50	23.08	29
Detected during hospitalization for ischemic stroke and TIA (%)	4.17	4.35	4.95	5.77	3.85	6.5
Newly detected at admission (%)	–	2	3.7	–	–	–
Stroke-related factors						
Stroke severity						
NIHSS median	7	7	11	9	12	8
Loss of consciousness (drowsy/comatose) (%)	35.9	25.9	21.25	32.7	21.67	25.7
Type of stroke						
Ischemic stroke (%)	91.14	78.38	60.78	83.33	86.67	84.5
Intracerebral hemorrhagic (%)	7.6	12.61	17.65	13.33	13.33	9.9
Subarachnoid hemorrhagic (%)	1.27	2.1	1.8	0	0	0

Note: AF – atrial fibrillation; TIA – transient ischemic attack; NIHSS – National Institutes of Health Stroke Scale or NIH Stroke Scale/Score.

Processes of Care

Almost all hospitalized patients with stroke received neuroimaging, so between 2017 – 2022 the percentage of brain CT scans did not change much (100% in 2022, 93.3% in 2021, 96.7% in 2020, 90% in 2019, 97.2% in 2018, and 98.72% in 2017), but a significant decrease in CT performed in the first hour after admission was noted (23.21% in 2021, 25.86% in 2020, vs 92.31% in 2018) (Fig. 1).

Among those with ischemic stroke, more than a half of patients received carotid investigation ≤7 days after hospital admission. Carotid artery imaging was less performed in the pandemic period (77.4% in 2022 and 65.38% in 2021 vs. 87.5% in 2017) (Fig. 2, Tab. 2).

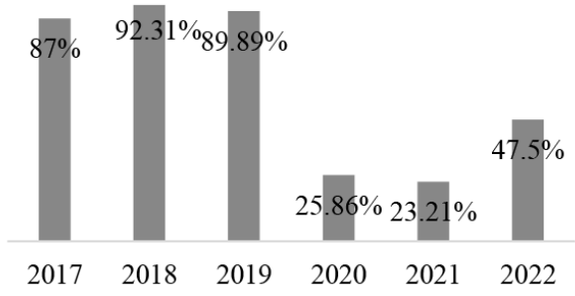

Fig. 1 CT performed within 1 hour after admission.

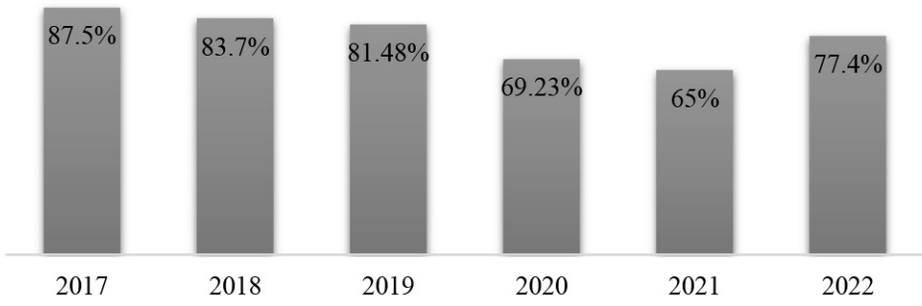

Fig. 2 The dynamics of carotid arteries imaging per year (%).

Carotid endarterectomy or angioplasty/stenting was done or planned for patients with ischemic stroke with internal carotid artery stenosis >50% among 33.33% of the patients with ischemic stroke eligible for the procedures in 2021 vs. 16.67% in 2019 (Tab. 2).

Table 2. *Processes of care of RES-Q Registrants.*

Year	2017	2018	2019	2020	2021	2022
Neuroimaging (%)	98.72	97.2	90	96.7	93.3	100
CT performed in the first hour after admission (%)	87.01	92.31	89.89	25.86	23.21	47.5
Carotid investigation ≤7 days after hospital admission for ischemic stroke (%)	87.5	83.7	81.48	69.23	65	77.4
Carotid endarterectomy or angioplasty/stenting done or planned for carotid stenosis >50% (%)	66.67	66.67	16.67	0	33.33	–
Intravenous thrombolysis (%)	2.78	4.6	11.29	10	7.69	20
Endovascular treatment (%)	–	–	3.23	–	5.77	10
Time to needle (minutes) (%)	85	93.5	53	25	48	51.5
Patients put on ventilator (%)	unknown	18.56	16.88	19.57	30.51	16.7
Assessment for rehabilitation needs ≤72 hours after hospital admission (%)	86.62	91.3	61.84	63.16	55	90.9
Dysphagia screening (%)	37.74	77.13	58	78.9	48.34	82.1
Antihypertensive agent (%)	98.44	97.8	92.86	95.56	89.47	96.9
Cholesterol-lowering medication (%)	58.33	56.99	44.44	44.23	59.09	60
Prescribed antiplatelets to patients without AF (%)	100	100	81.63	100	92	88.9
Anticoagulants in AF (%)	70.83	100	35.71	71.43	100	55

Note: AF – atrial fibrillation; CT – computed tomography scan.

The number of recanalized patients was not affected by COVID-19 pandemic (21.7% in 2022, 13.46% in 2021 and 10% in 2020 vs. 14.62% in 2019, 4.6% in 2018 and 2.78% in 2017) (Fig. 3). The proportion of patients with ischemic strokes receiving thrombolysis therapy has substantially increased from 2.78% in 2017 to 20% in 2022, and the median door-to-needle time decreased from 85 minutes (2017) to 48 minutes (2021) (Fig. 4). Thrombectomy began to be performed in 2018 at the Institute of Emergency Medicine, the proportion of patients with ischemic strokes receiving

thrombectomy increased from 3.23% in 2019 to 10.0% in 2022, and the median door-to-groin time decreased from 228.5 minutes in 2019 to 102 minutes in 2021.

All these data demonstrate the effort to improve the quality of stroke care at this hospital.

Approximately two-thirds of the participants in 2021 and 90.9% in 2022 had assessments for rehabilitation needs ≤72 hours after hospital admission. Almost a third of patients (37.74%) in 2017, three quarters of patients (78.9%) in 2020 and in 2022 (82.1%) with stroke received dysphagia assessment or screening. In general, more than 80% of patients received secondary prevention therapies that were level-1 evidence-based processes of care: antihypertensive agents (89.47% – 98.44%), antiplatelet drugs (81.63% – 100%), anticoagulants for patients with atrial fibrillation (70.83% in 2017 vs. 100% in 2021), but cholesterol-lowering medication was prescribed to approximately a half of the participants (44.23% – 60.0%). There were no differences between men and women in the care received (Tab. 2).

In the pandemic period, there were more patients put on ventilator: 19.57% in 2020, 30.51% in 2021, vs. 18.56% in 2018 and 16.88% in 2019 (Tab. 2).

There were more deaths in the pandemic years (36.67% in 2021, 23.33% in 2020, vs. 14.71% in 2019) (Tab. 3, Fig. 5).

Table 3. *Discharge destination.*

Home (%)	67.09	80.18	80.39	68.33	51.67	78.3
Rehabilitation facility (%)	13.92	3.6	4.9	8.34	8.33	9.1
Other hospital (%)	0	0	0	0	3.33	0.2
Died (%)	18.99	16.22	14.71	23.33	36.67	12.4
Median hospital stay	9	10	10	8	8	10

An important part of centralized stroke care is also rehabilitation (during the acute disease phase as well as long-term rehabilitation). In 2021, 55% of patients benefited from rehabilitation measures during inpatient treatment, with the majority of stroke patients being subsequently discharged at home (51.67%), only 8.33% of patients being transferred for rehabilitation purposes to another center, and 36.67% died (Tab. 2). So, in 2020 – 2021 the pandemic had a predominantly negative impact on the diagnostic process and on the evolution of stroke, but an improvement in the quality of stroke management during the 2022 year is remarkable: 90.9% of patients benefited from rehabilitation measures, with the majority of stroke patients being subsequently discharged at home (78.3%), only 9.1% of patients being transferred for rehabilitation purposes to another center, and 12.4% died (Tab. 3).

Discussion

The number of recanalized patients was not affected by COVID-19 pandemic (21.7% in 2022, 13.46% in

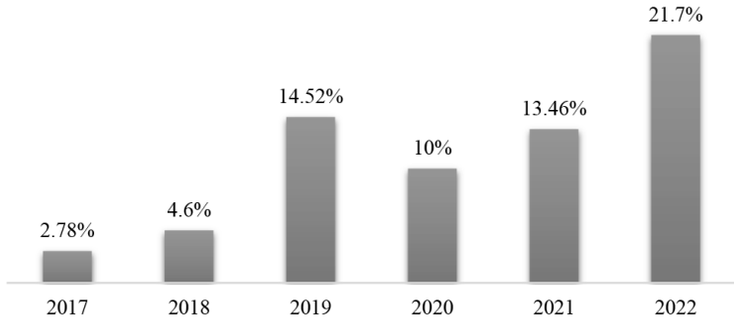

Fig. 3 The dynamics of recanalized patients per year (%).

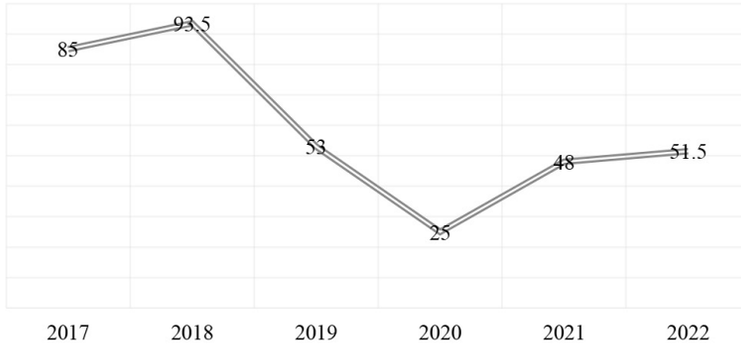

Fig. 4 The dynamics of door-to-needle time per year (minutes).

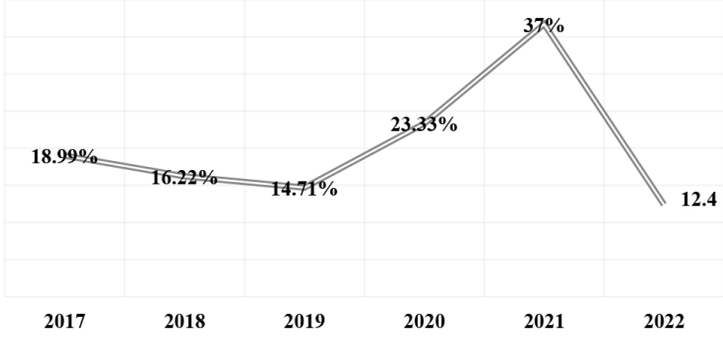

Fig. 5 Stroke deaths.

This figure presents the dynamics of deaths for the years 2017 – 2022. There were more deaths in the pandemic years (36.67% in 2021, 23.33% in 2020, vs. 14.71% in 2019).

2021 and 10% in 2020 vs. 14.62% in 2019, 4.6% in 2018 and 2.78% in 2017). The proportion of patients with ischemic strokes receiving thrombolysis therapy has substantially increased from 2.78% in 2017 to 20% in 2022, and the median door-to-needle time decreased from 85 minutes (2017) to 48 minutes (2021).

Thrombectomy began to be performed in 2018 at the Institute of Emergency Medicine; the proportion of patients with ischemic strokes receiving thrombectomy increased from 3.23% in 2019 to 10.0% in 2022; the median door-to-groin time decreased from 228.5 minutes in 2019 to 102 minutes in 2021, demonstrating the effort to improve the quality of stroke care at this hospital.

The considerable delay in time from stroke onset to hospital arrival remains an important issue. More than 70% of those with stroke admitted to our hospital did not arrive ≤4.5 hours after experiencing stroke symptoms, and therefore they were potentially ineligible for intravenous thrombolysis therapy. The median door-to-needle time at the hospital, which has decreased to 48 minutes in 2021 and 51.5 minutes in 2022, has met the American Heart Association/American Stroke Association's initial goal (≤60 minutes for at least 50% of acute ischemic strokes patients) [14, 15].

Some aspects of care, such as assessment for dysphagia screening, AF screening, rehabilitation needs, and carotid investigation, appeared to be less than optimal. The reasons for the lack of assessment for dysphagia screening, AF screening, rehabilitation needs and carotid investigation after hospital admission included the patients' condition, which did not allow for assessment, as well as lack of personnel and equipment, such as Holter ECG for AF screening. Between 2017 and 2022, AF was detected during hospitalization for ischemic stroke and TIA in 3.85% – 6.5% patients by electrocardiogram. Holter ECG is an important tool for detecting cardioembolic strokes and for optimal secondary prevention. It is recommended for all ischemic strokes where the etiology is not clearly identified. One of the indicators of the RES-Q database is the percentage of the Holter ECG indication for those types of ischemic stroke.

The data collection of indicators of stroke care quality brings feedback to each stroke center and controls its activity and results. The determination of the type of an ischemic stroke is important, especially for secondary prevention [16]. This is related to the implementation of ECG Holter monitoring for ischemic strokes. The detection of swallowing disorder is also important for the prevention and treatment of subsequent complications as well as for nursing care.

The occurrence of infection complications is an important issue during stroke care. A good example of personalized medicine in cerebrovascular diseases is the prediction of the risk of infection-related complications developing in an individual stroke patient [17]. The incidence of infectious complications during stroke treatment is not part of the RES-Q registry. However, infectious complications are required to be assessed as a part of hospital nosocomial infection monitoring.

There are currently more than 70 hospitals across the Republic of Moldova [18]. There are limited resources and infrastructure for stroke care and treatment, including a shortage of staff with expertise in stroke or who are up skilled to manage stroke according to best practice in hospitals. No more than 3 stroke units are available to service a population of more than 2.6 million [19, 20], suggesting that the vast majority of patients may not receive adequate evidence-based care. Therefore, there is an urgent need for more stroke units, and improved management of stroke as a medical emergency, and greater provision of specialized care across the country.

In 2021, we successfully implemented the new RES-Q Comprehensive form, which collects information about: (1) risk factors, medication, the modified Rankin Scale score (mRS), blood glucose, blood pressure, and cholesterol on admission; (2) antidotes for oral anticoagulants, rtPA dosage, and the TICI score; (3) brain imaging details (ASPECTS score, CTA/MRA occlusion, and CT/MR perfusion deficit), brain imaging after 24 hours and detection of hemorrhages/infarcts; (4) physiotherapy, ergotherapy, NIHSS at discharge and mRS at 90 days. These data will allow us to better understand the risk factors for stroke, to better reflect needs in understanding modern treatments for stroke, and to assess the clinical outcomes by the most widely used disability score – modified Rankin Scale (mRS) score at hospital discharge and at 3 months (90 days) following hospital discharge. Documentation of a mRS obtained within the 90-day timeframe (75 to 105 days after hospital discharge) via telephone or in-person is acceptable. If the patient cannot be interviewed because of communication deficits or other limitations, an interview with the patient's caregiver is acceptable.

Good management of risk factors can lead to dramatic changes in the incidence of stroke, and RES-Q Comprehensive form offered the possibility to study some of them [21]. The mean age of stroke patients in 2021 was 66.73±11.45 years old, and in 2022 it was 67.71±10.55 years old. In 2021, diabetes mellitus was noticed in 31.25% of patients, and only 63.2% of diabetic patients were receiving antidiabetic treatment, but in 2022, there were 28.2% of diabetic patients, and 65% were receiving treatment. In 2021, dyslipidemia was noticed in 46.85% of subjects (vs. 15.5% in 2022), congestive heart failure in 28.13% (vs. 14.1% in 2022), 23.08% had AF (vs. 29% in 2022), and 20.2% were smokers (vs. 7.04% in 2022). 6.25% of AF patients were receiving warfarin, and 15.62% received rivaroxaban in 2021, but the next year, warfarin was recommended for 10.5% of patients, and rivaroxaban for 11.0%. 81.25% of patients were receiving aspirin treatment at stroke onset. In 2021, the mean values of systolic and diastolic blood pressure at admission were 148.33±37.45 mmHg and, respectively, 85.55±23.64 mmHg, but in 2022, they were 166.05±30.46 mmHg and, respectively, 92.39±16.08 mmHg. In 2021, the mean value of blood sugar at admission was 7.62±3.59 mmol/l, but in 2022, it was 7.97±3.16 mmol/l, and the mean value of serum cholesterol was 5.05±1.02 mmol/l and, re-

spectively, in the following year, it was 4.51±1.54 mmol/l. In 2021, 23.33% of stroke patients were tested for COVID-19, while in 2022 a smaller number (8.5%) were tested. A rate of 16.4% of patients in 2021, and a rate of 8.5% of patients in 2022 had repeated strokes.

In 2022, it was found that the average period from the onset of the disease to admission to the hospital was 249.5±275.01 minutes, and only 33.8% of patients with stroke were admitted during the therapeutic window period.

Conclusions

Most hospitalized patients with acute stroke admitted to the Institute of Emergency Medicine, Chisinau, Republic of Moldova, received evidence-based care. The COVID-19 pandemic has left its mark on the number of stroke hospitalizations, but the quality of in-hospital stroke care was not dramatically affected. However, the considerable delay in time from stroke onset to hospital arrival remains an important issue. More than 70% of those with stroke admitted to our hospital did not arrive in less than 4.5 hours after experiencing stroke symptoms, and they were then potentially ineligible for intravenous thrombolysis therapy.

List of abbreviations used

RES-Q – the Registry of Stroke Care Quality; AF – atrial fibrillation; TIA – transient ischemic attack; CT – computed tomography; CTA – computed tomography angiography; MRA – magnetic resonance angiography; ECG – electrocardiogram; NIHSS – National Institutes of Health Stroke Scale; mRS – modified Rankin Scale score.

Conflict of interests

Nothing to declare.

Authors' contributions

SG interpreted the data, drafted the manuscript, and revised the manuscript critically; NC collected the data, made statistical analysis, drafted the manuscript, and interpreted the data; IC interpreted the data and drafted the manuscript; SP interpreted the data and drafted the manuscript; EZ revised the manuscript critically; IS collected the data. All the authors revised and approved the final version of the manuscript.

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RESEARCH ARTICLE

Cardiocytoprotection with metabolic drugs - study of the effectiveness of meldonium in ischemic heart disease

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What is not yet known on the issue addressed in the submitted manuscript

From the point of view of evidence-based medicine, in the treatment of effort angina, priority is given to drugs, which have a level of proof of efficacy in class I and IIa, and an attempt to increase the effectiveness of complex treatment by introducing metabolic pharmacotherapy into clinical practice in order to ensure cardiocytoprotection.

The research hypothesis

After the administration of meldonium, a significant reduction in the level of malonic dialdehyde in erythrocytes was observed, which is a sign of a significant decrease in the degree of tissue hypoxia. A significant decrease in serum concentrations of organ-specific myocardial enzymes was also detected.

The novelty added by manuscript to the already published scientific literature

This randomized controlled clinical trial of patients with angina pectoris of stable effort has found a 4-fold increase in the effectiveness of complex pharmacotherapy in ischemic heart disease when adding meldonium compared to the basic treatment because of antianginal effect, improvement of physical performance, potentiation of the positive inotropic and hypotensive effects of basic pharmacotherapy.

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Summary

Introduction. Ischemic heart disease is one of the most widespread cardiovascular diseases. In Republic of Moldova the total number of patients with ischemic heart disease is 30-40 thousand per 1 million population and is observed more of working age with important social value.

Materials and method. An open randomized clinical trial involving 139 patients with chronic heart failure (107 men and 32 women) aged 37 to 81 years: 111 patients had angina pectoris of stable effort from different functional classes, and 28 – unstable angina pectoris, which include: basic treatment n = 43; basic treatment + meldonium (non-infarct) n = 52; basic treatment + meldonium (post-infarct) n = 35; basic treatment + meldonium, (aggravated) n = 9. Study groups were compared according to the frequency of using background (basic) drugs and meldonium. Statistical processing of the results was carried out in Statistics Software Package 9.0.

Results. During the treatment, the increase of the nitric oxide level was registered even from the discharge stage, and in the second group being approximately at the same level as the initial stage. At the 3-month, nitric oxide level reached the normal level. There is an improvement of the endothelial dysfunction by the significant increase of the nitric oxide under the treatment at 6 months (in group I – 87.26±4.3 μM/L (p = 0.01), in group II – 95.33±10.85 μM/L).

Conclusions. The inclusion of meldonium in the complex treatment of patients with stable angina increases the clinical efficacy of basic pharmacotherapy when prescribing meldonium, mainly due to increased antianginal actions.

Keywords: cardiocytoprotector, cardiac metabolism, ischemic heart disease.

Introduction

Diseases of the cardiovascular system in most countries of the world are on the first place among the causes of deaths [1-3]. The epidemiological situation in the Republic of Moldova is characterized by the term „supermortality" due to cardiovascular diseases, compared to economically developed countries [2-4]. Ischemic heart disease (IHD) is one of the most widespread cardiovascular diseases [5]. In our country, the total number of patients with ischemic heart disease is 30-40 thousand per 1 million population [2, 4, 6]. Nowadays, a process of "rejuvenation" of the CHF is observed, the hospital beds being occupied more often by patients of working age, occupying important positions in society, which is why this disease needs to be considered as one with important social value.

In the current time, based on the results offered by multicenter studies on the efficacy of contemporary drugs, international and national guidelines have been developed for the treatment of stable exertional angina pectoris [5, 7]. From the point of view of evidence-based medicine, in the treatment of exertional angina, priority is given to drugs, which have a level of proof of efficacy in class I and IIa, from the groups of antiplatelets and anticoagulants, beta-blockers, statins, angiotensin-converting enzyme inhibitors; drug forms from other groups (nitrates, calcium antagonists) can also be used, but have less influence on the indices of survival and life span of patients [6, 8, 9].

An attempt to substantially increase the effectiveness of complex treatment of ischemic heart disease is the introduction into clinical practice of metabolic pharmacotherapy with the aim of providing cardiocytoprotection [7-10].

The purpose of the study was to study the effectiveness and safety of the metabolic drug meldonium in ischemic heart disease.

Material and methods

Our study included 160 patients with CHF (107 men and 32 women) aged 37 to 81 years. Of them, 139 patients had angina pectoris of stable effort from different functional classes, and 20 – unstable angina pectoris. In most patients, angina pectoris was associated with hypertension (HTA) (143 [89.4%]), rhythm disturbances (39 [24.4%]), postinfarct cardiosclerosis (CSPI) (78 [48.8%]), chronic heart failure (CHF) (151 [94.4%]), some with diabetes mellitus (DM) type II (37 [23.1%]). The average age was 59.26±0.74 years. All patients were divided into 2 groups – with and without using meldonium and consist of: n = 43 only with background treatment; non-infarct IHD (n = 52) with background treatment and meldonium, post-infarct IHD (n = 35) with background treatment and meldonium and n = 9 with aggravated IHD who were treated with background treatment and meldonium. The observation period was 6 weeks. Each participant was introduced to the research program and signed an informed agreement (a favorable decision of Ethics Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy nr. 17 from 10th of April 2012).

Meldonium was administered at a dose of 0.5 g/24 hours for a period of 6 weeks: in the first 10 days, the preparation was administered intravenously in the hospital setting, after which patient continued the treatment with capsules as an outpatient. Study groups were comparable according to the frequency of indication of background drugs.

Table 1. *Frequency of indication of background remedies in study groups of patients with stable exertion angina pectoris*

Group of medicinal remedies	Study groups			
	Background treatment, n = 43	Background treatment + meldonium, (non-In) n = 52	Background treatment + meldonium, (post-In) n = 35	Background treatment + meldonium, (Aggravated) n = 9
Disaggregating drugs	43 (100%)	52 (100%)	35 (100%)	9 (100%)
Statins	27 (62%)	32 (61%)	23 (65%)	6 (65%)
Beta-blockers	34 (80%)	41 (79%)	30 (86%)	8 (84%)
ACE inhibitors	34 (80%)	40 (77%)	27 (78%)	7 (78%)
Calcium antagonists	18 (42%)	21(40%)	15 (43%)	4 (41%)
Prolonged nitrates	27 (63%)	30 (58%)	21 (59%)	5 (57%)

Note: ACE – angiotensin-converting enzyme; non-In – non-infarction; post-In – postinfarct

Results

The clinico-experimental research of meldonium in myocardial ischemia was investigated in patients with a diagnosis of ischemic heart disease: angina pectoris of stable effort functional class (CF) I-IV, postinfarct cardiosclerosis (in 17 [48%] patients) in combination with hypertension stage II-III, grade 2-3, complicated with chronic heart failure (CHF) stages I-II A, CF I-III NYHA, in 8 (23%) patients with concomitant type II diabetes mellitus. The control group consisted of 43 patients who received only basic treatment.

At the addition of meldonium, there was an improvement on the ECG in the repolarization phase in the form of reducing the depth of the negative wave „T" from 1.5 mm to 0.2 mm ($p < 0.05$) and the decrease in the number of negative wave derivatives „T" from 2.6 to 0.4 ($p = 0.07$), compared to the basic therapy those indices did not undergo significant changes. At the end of the observation period, patients treated with meldonium were able to walk in 6 minutes 166 meters more than before treatment, increasing their result from 310.66±24.74 meters to 476.50±43.5 meters ($p < 0.05$), while patients who received only basic treatment, were able to walk 352.45±18.28 meters to treatment, and at the end of the observation period practically as much as 365.00±5.00 meters ($p > 0.05$). The addition of meldonium offered an additional hemodynamic effect in the form of a significant decrease in blood pressure – systolic from 161.76±4.39 mmHg to 143.45±5.13 mmHg ($p < 0.05$) and diastolic from 95.09±2.88 mmHg to 87.54±2.52 mmHg ($p = 0.06$). The summary coefficient of effectiveness of the basic therapy made up 15.55±4.21%, and of complex pharmacotherapy with meldonium 59.16±3.31% ($p < 0.001$), which in fact is 4 times higher. In order to elucidate the mechanism of action of meldonium on energy exchange in cardiomyocytes, an extensive study was carried out. Meldonium administration induced a significant increase in the concentration of adenosine triphosphate (ATP) in blood serum and erythrocytes practically to the optimal level, suggesting the elimination of energy deficiency caused by ischemia (table 2).

Table 2. *Indicators of myocardial metabolism in the norm, in non-infarct and post-infarct ischemic heart disease (M±m)*

	Indicators	Norm	Background treatment	Background treatment + Meldonium
Erythrocytes	ATP, μmol/l	664.54±14.49$^{\Delta\Delta}$	594.44±5.75**$^{\$}$	638.88±14.96$^{\Delta}$
	ADP, μmol/l/l	315.11±8.78	330.53±16.05	319.27±7.51
	2,3-DAM, μmol/l/l	4.82±0.29$^{\Delta\Delta\$}$	7.21±0.32**$^{\$\$}$	5.70±0.8*$^{\Delta\Delta}$
Blood serum	ATP, μmol/l/l	200.08±3.47$^{\Delta\Delta}$	162.81±4.57**$^{\$\$}$	192.32±5.36$^{\Delta\Delta}$
	ADP, μmol/l/l	75.92±1.58	79.31±1.13	77.33±2.29
	Pyruvate, μmol/l/l	58.59±2.26	59.95±1.02$^{\$}$	53.44±1.99$^{\Delta}$
	Lactate, μmol/l/l	0.50±0.03	0.62±0.14$^{\$\$}$	0.59±0.03
	CFK-MB, μmol/l	0±0$^{\Delta\Delta}$	0.25±0.04**$^{\$\$}$	0±0$^{\Delta\Delta}$
	LDH_1, μmol/l	0.02±0.00$^{\Delta\Delta\$\$}$	0.09±0.01**$^{\$\$}$	0.05±0.01**$^{\Delta\Delta}$
Mitochondria	SOD, nmol/min	17.82±1.10$^{\Delta\Delta\$}$	11.83±0.47**	13.68±0.65*
	CAT, nmol/min	3.94±0.23$^{\Delta\Delta\$}$	2.38±0.21**$^{\$}$	3.12±0.10*$^{\Delta}$
	PDH, μmol/l	31.04±0.89$^{\Delta\Delta\$\$}$	21.68±0.90**$^{\$\$}$	27.30±0.45**$^{\Delta\Delta}$

Note. The statistical significance of the differences was evaluated by the modified t-Student criterion with the Bonferroni correction: * – $p < 0.05$; ** – $p < 0.01$ compared to the optimum value; $^{\Delta}$ – $p < 0.05$; $^{\Delta\Delta}$ – $p < 0.01$ compared to the group with ischemic heart disease; $^{\$}$ – $p < 0.05$; $^{\$\$}$ – $p < 0.01$ compared to the group with ischemic heart disease + meldonium.

DAM – malonic dialdehyde; ATP/ADP – Adenosine triphosphate and diphosphate; CFK – creatine phosphokinase; LDH – lactate dehydrogenase; SOD – superoxide dismutase; CAT – catalase; PDH – pyruvate dehydrogenase.

Compared with the background treatment, the addition of meldonium was associated with a significant reduction in the level of malonic dialdehyde (DAM) in erythrocytes, a sign of a significant decrease in the degree of tissue hypoxia. A significant decrease in serum concentrations of organ specific myocardial enzymes – creatinine phosphokinase (CFK) and lactate-dehydrogenase (LDH1) has been detected, which speaks of reducing the „leakage" of enzymes from the cytoplasm of cells following the stabilization of cardiomyocytic membranes. A significant decrease in the plasma concentration of pyruvate was detected, and in the mitochondria – activation of pyruvate-dehydrogenase (PDH), indicating the stimulation of the oxidative decarboxylation process of pyruvate. In addition, in the mitochondria, a significant activation of catalase (CAT) and insignificant activation of superoxide-dismutase (SOD) was revealed. These data suggest the slight stimulation of the processes of oxidative phosphorylation, which, on the one hand, provides the cell with energy, and on the other hand, uses only the oxygen that is available, without increasing the need in conditions of tissue hypoxia. We can say that meldonium acts quite harmoniously, activating the aerobic processes of energy intake to cardiomyocytes according to the degree of reduction of tissue hypoxia. In addition, signs of activation of anaerobic mechanisms for extracting energy from carbohydrates by stimulating the glycolysis process, as evidenced by the increase in CFK activity and the increase in lactate capture by the myocardium have been detected. Thus, in patients with myocardial ischemia meldonium activates glycolysis, oxidative phosphorylation and oxidative decarboxylation, stabilizes the cardiomyocyte membrane, significantly reduces the degree of hypoxia, which leads to the restoration of the initial level of ATP and adequate energy intake to the myocardium.

Until the initiation of the treatment, a compromised antioxidant system is attested in patients from all groups statistically significant ($p<0.001$) (table 2). The antioxidant system was activated from the first hours after the initiation of the treatment. At the initial stage, the SOD level showed no significant differences between the groups. At the period of 1, 3, 6 months, increased SOD values were recorded. Thus, at 6 months, the SOD level reached 11.83±0.47 μmol/l in group I and 13.68±0.65 μmol/l in group II. At 12 months the SOD level decreased, statistically significant ($p<0.01$) in group II. During the treatment, the increase of the NO level was registered even from the discharge stage, and in the second group being approximately at the same level as the hospitalization stage (50.62±2.84 μmol/l vs 50.03±2.25 μmol/l). At 3 months, the practical NO level reached the reference level of group I (78.51±7.0 μmol/l vs. 78.66±2.72 μmol/l), in group II there was an insignificant increase compared to the initial stage (64.70±9.13 μmol/l vs. 50.62±2.84 μmol/l). There is an improvement of the endothelial dysfunction attested by the significant increase of nitric oxide at 6 months: in the I group – 87.26±4.3 μmol/l ($p = 0.01$), in the II group – 95.33±10.85 μmol/l. All levels are higher than the initial ones.

Discussion

The analysis of the peculiarities of the influence of meldonium on the metabolism of cardiomyocytes in myocardial ischemia in young and old patients leads to the idea of a striking harmony. The explanation of the obtained results may be due to the mechanism of action of meldonium. This drug blocks carnitine biosynthesis from the butyrobetain range, causing a double positive effect [3-6]. First, it reduces the concentration of carnitine, a transporter of fatty acids through the mitochondrial membrane, which causes energy-saving effects [2, 4, 10]. Secondly, it increases the concentration of gamma-butyrobetaine, which excites acetylcholine receptors and stimulates nitric oxide biosynthesis – the mediator of the

STRESS-limiting NO-ergical system, universal regulator of the adaptation process [2, 5, 9]. In clinical trials, the ability of meldonium to provide an adaptogenic effect by regulating the biosynthesis of NO has been demonstrated [1-8]. Probably this mechanism has a certain contribution to the realization of such a harmonious influence of the preparation on the metabolism of cardiomyocytes in the conditions of myocardial ischemia in both young and old patients.

Limiting the adaptive capacities of cells to restore their own energy and plastic resources in old age reduces the possibilities of meldonium to corrode metabolism in myocardial ischemia in elderly patients. The mechanisms of action of meldonium revealed in the experiment explain in many aspects the positive effects of this drug that we observed in the clinic.

In 10 (10.4%) of the patients examined by us, the monovascular damage of the coronary circuit was detected, in 9 (9.4%) – bivascular and in 72 (75.0%) – polyvascular. The trunk of the left coronary artery was affected in 37 (28.5%) of patients with the average stenosis degree of 17.92±2.67%, the anterior interventricular artery – in 82 (85.4%) patients with the average degree of stenosis of 57.32±3.33%. The same were described the circumflex artery at 67 (69.8%) with the average degree of stenosis of 44.01±3.71%, the intermediate artery at 23 (24.0%) patients with the average degree of stenosis of 14.89±3.01%. Besides that, we may mention the fact that the right coronary artery – in 67 (69.8%) patients with the average degree of stenosis of 46.41±3.95% and the posterior interventricular artery – in 19 (19.8%) patients with the average degree of stenosis of 12.11±2.81% were affected.

Thus, in our patients with angina pectoris of stable effort, a significant 4-fold increase in the effectiveness of complex pharmacotherapy in ischemic heart disease was found when adding meldonium compared to the basic treatment on account of the more pronounced antianginal effect. The improvement of physical performance and potentiation of the positive and hypotensive inotropic effects of basic pharmacotherapy were observed in groups with meldonium. According to experimental data in patients with myocardial ischemia meldonium activates glycolysis, oxidative phosphorylation and oxidative decarboxylation, stabilizes the cardiomyocyte membrane, significantly reduces the degree of hypoxia, thereby restoring the initial level of ATP and achieving adequate energy intake of the myocardium. This drug quite harmoniously manages the metabolism of cardiomyocytes in conditions of experimental myocardial ischemia given the initial energy status, the degree of tissue hypoxia and the age of the patients.

Therefore, the results of our study indicate the activation of oxidative stress in patients with stable angina pectoris, relevant in this regard being the changes of DAM, catalase and SOD, which become more pronounced in the first 24 hours after starting the treatment and although by month 6 a decrease in the activity of the prooxidant status is detected, it intensifies at 12 months. Patients with more pronounced deviations of oxidative stress markers have a higher risk of developing IHD. This completes the vision based on the link between the antioxidant defense and the aggravated cardiovascular evolution. Another consolidated aspect is to demonstrate the superior effectiveness of meldonium administration. For the first time in the Republic of Moldova it was demonstrated the feasibility of the effectiveness of meldonium vis-à-vis the markers of oxidative stress, endothelial dysfunction and comparable systemic inflammation. The worldwide experience of using meldonium is very limited, therefore the results obtained have a conclusive impact on the accumulation of evidence in this regard.

Conclusions

The inclusion of metabolic drugs in the complex treatment of patients with stable angina increases the clinical effectiveness of basic pharmacotherapy by 4 times when prescribing meldonium (59.16% compared to basic therapy 15.95%, $p < 0.001$), mainly due to increased antianginal actions.

Using of meldonium in patients with myocardial ischemia leads to the accumulation of ATP inside cardiomyocytes due to the activation of various bonds of energy metabolism: meldonium activates anaerobic glycolysis, oxidative phosphorylation and oxidative decarboxylation of pyruvate, leading to a complete restoration of the amount of ATP in the myocardium. The introduction of meldonium metabolic corrector in patients with coronary heart disease is accompanied by stabilization of the membranes of cardiomyocytes and a decrease in the degree of tissue hypoxia. In elderly patients, meldonium retains its activity in the best possible way.

Abbreviations

ATP/ADP – adenosine triphosphate and diphosphate; CAT – catalase; CF – functional class; CFK – creatine phosphokinase; CHF – chronic heart failure; CSPI – postinfarct cardiosclerosis; DAM – malonic dialdehyde; DM – diabetes mellitus; ECG – electrocardiogram; HTA – hypertension; IHD – ischemic heart disease; LDH – lactate dehydrogenase; NO – nitric oxide; PDH – pyruvate dehydrogenase; SOD – superoxide dismutase.

Declaration of conflict of interest

Nothing to declare

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RESEARCH ARTICLE

Risk assessment of pericoronitis in correlation with the position of the inferior third molar

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What is not known yet about the topic

The predisposing factors in relation to the etiology of inferior wisdom molar pericoronitis are still a controversial topic in the literature data. One of the most discussed hypotheses is that the position of the wisdom molar could favor the development of the inflammatory-infectious process. Currently, however, there is insufficient data to reveal which atypical orientations of the lower wisdom molar cause an increased risk of developing pericoronitis.

Research hypothesis

The vertical and mesio-angular position (by Winter classification) predispose to a high risk of developing pericoronitis.

The novelty added by manuscript to the already published scientific literature

Based on the obtained results, we will have a better understanding about the predisposing factors for the inflammatory-infectious processes of the inferior molar surrounding tissues.

Abstract

Introduction. The inferior third molar is the most encountered impacted permanent tooth. Pericoronitis of the lower third molar is a term used to describe the inflammation around the crown of a tooth, usually of an incompletely erupted mandibular third molar. Mandibular molar impactions are usually mesioangular, distoangular, vertical, and horizontal. The position and type of the impaction may affect the development of pericoronitis. This study was conducted to assess the positions of the lower wisdom tooth as a risk factor for pericoronitis.

Material and methods. The present study was conducted in the department of Oral and Maxillofacial Surgery and Oral Implantology „Arsenie Guțan". A number of 120 patients were included in the study (66 women and 54 men) aged between 17-46 years old (mean 27±SD 6). All candidates were subjected to CBCT (cone beam computed tomography) scan and orthopantomography evaluation for a third molar position such as vertical, mesioangular, distoangular, and horizontal type. Data were stored in an Excel spreadsheet and then analyzed statistically using SPSS (Statistical Package for the Social Sciences).

Results. Based on the results of our study the greatest number of cases of pericoronitis was observed in the vertical position (92 cases) by Winter, followed by mesioangular position (24 cases) and distoangular position (4 cases). In the study we did not identify any patient with a horizontal position. By the classification of Pell and Gregory, most cases belonged to class I (73 cases). Third molars classified in position A (92 cases) had greater chance of pericoronitis when compared to those in B (24 cases) or C position (4 cases).

Conclusions. As a result of a detailed analysis, vertical position of the lower third molar is more associated with the appearance of pericoronitis. Considering Pell and Gregory classification, position A is more associated with the occurrence of pericoronitis compared to the position B or C. The prophylactic removal of the lower third semi-erupted vertical molar, or which is situated in position A, is indicated to prevent pericoronitis.

Key words: Pericoronitis, inferior third molar, Winter, Pell&Gregory.

Introduction

The mandibular third molar is the most frequent impacted permanent tooth [1]. Various studies show a prevalence of 9.5 to 39% of third mandibular molars that fail to erupt into the oral cavity [2]. Mandibular molar im-

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pactions are usually mesioangular, distoangular, vertical, and horizontal. The position and type of impaction may affect the development of pericoronitis [3]. Pericoronitis is an inflammatory and infectious condition that usually affects an incompletely erupted mandibular third molar. The localized inflammation may range from major regional reactions, such as cellulitis, trismus, pain, and bleeding, to general reactions, such as fever and asthenia [4]. Studies show that pericoronitis is the most common acute problem associated with third molars, and there are several predisposing factors related to the etiology of the disease. One of them is the position of impaction; thus, understanding the relationship between the occurrence of pericoronitis and the clinical and radiographic conditions of the lower third molars is the key for the determination of the clinical behavior of these teeth [5]. The status of impaction can be judged radiographically on orthopantomography and cone beam computed tomography (CBCT). This kind of investigation provides information on assessing the position and status of the tooth. The aim of this study is to determine the most common positions of impaction of the lower wisdom teeth as a risk factor for pericoronitis.

Materials and methods

The present study was conducted in the department of Oro-Maxillo-Facial Surgery and Oral Implantology „Arsenie Guțan" and involved 120 (66 women and 54 men) patients with an age range of 17 – 46 years (mean 27±SD 6), who came for dental surgical treatment during 2017–2022.

The study was approved by the Ethics Committee of the Nicolae Testemițanu State University of Medicine and Pharmacy, No.82 from 26.04.2017 and informed consents were obtained from all subjects involved in the study.

Patients with partially erupted third molars and radiographic evidence of impaction were included in the study. Clinical conditions such as redness, pus or exudate, pain, trismus, and sensitivity over the pericoronal flap have been evaluated. During the radiological examination, all participants were subjected to CBCT in all three planes scan, operating at 120 kVp, 15 mA, and 18s, and Ortopantomography scan operating at 120 kVp, 15 mA, and 18s, taken with a Planmeca machine. To classify the third molar position of impaction, we used the Winter classification (Figure 1). Reported to the second molar axis, we classified it into vertical (10°), mesioangular (+11°–70°), distoangular (−11°–70°), and horizontal (>70°) positions. All obtained images were read by three independent doctors to increase the accuracy of the study.

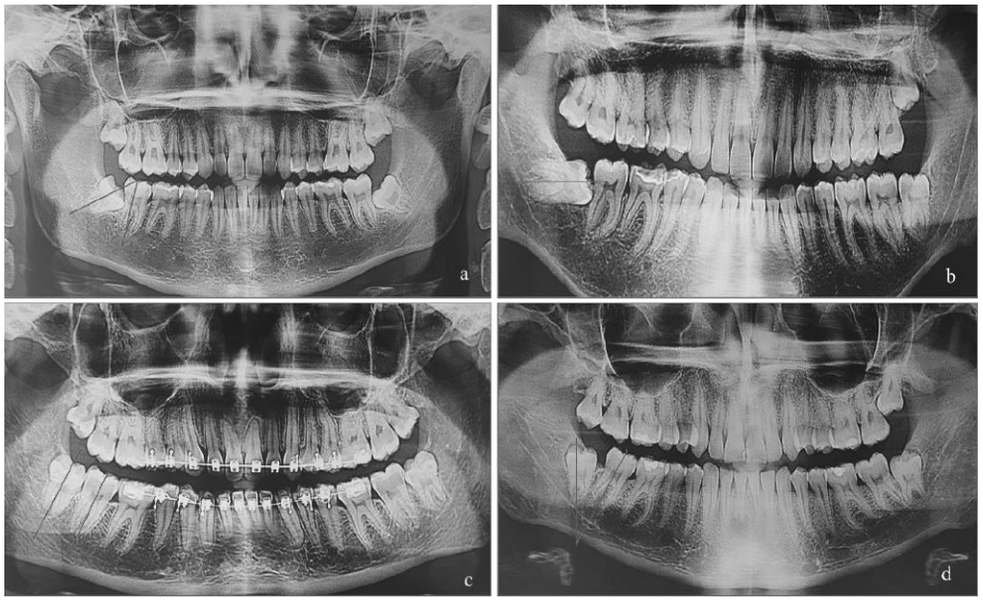

Fig. 1 Third molar impaction by Winter classification (OPG).
(a) – mesio-angular impaction, (b) – horizontal impaction, (c) – vertical impaction, (d) – disto-angular impaction.

Obtained results were subjected to statistical analysis using the SPSS statistical software (IBM SPSS) version 26.0. Independent ttest and Chisquare test were used to determine the significant association between the two variables. P value < 0.05 was considered significant.

Results

Table 1 shows that in our study we involved 120 patients (66 women and 54 men) with an age range of 17 – 46 years (mean 27±SD 6). Table 1 shows that the maximum number of cases of pericoronitis was observed in the age group 21–25 years (53 patients), followed by 26 – 30 years in 30 cases, and 31 – 35 years in 15 cases. The result was statistically significant at P < 0.05

Table 1. Classification of patients by age group.

Age, groups	<= 20 years	Total	10
		Column N %	8,3%
		95.0% Lower CL for Column N %	4.4%
		95.0% Upper CL for Column N %	14.3%
	21 – 25 years	Total	53
		Column N %	44.2%
		95.0% Lower CL for Column N %	35.5%
		95.0% Upper CL for Column N %	53.1%
	26 – 30 years	Total	30
		Column N %	25.0%
		95.0% Lower CL for Column N %	17.9%
		95.0% Upper CL for Column N %	33.3%
	31 – 35 years	Total	15
		Column N %	12.5%
		95.0% Lower CL for Column N %	7.5%
		95.0% Upper CL for Column N %	19.3%
	36 – 40 years	Total	9
		Column N %	7.5%
		95.0% Lower CL for Column N %	3.8%
		95.0% Upper CL for Column N %	13.2%
	>=41 years	Total	3
		Column N %	2.5%
		95.0% Lower CL for Column N %	0.7%
		95.0% Upper CL for Column N %	6.5%

Note: CL – confidence limit; N – number; Upper 95% confidence limit (CL) – sample mean + (1.96); Lower 95% CL – sample mean – (1.96).

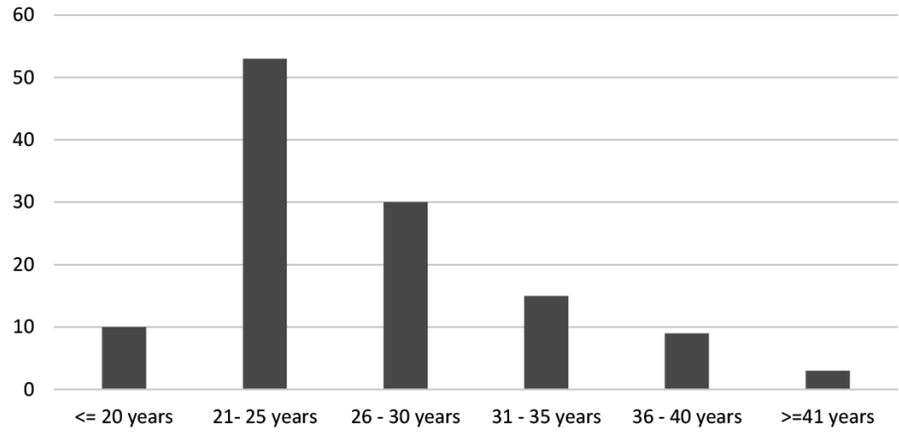

Fig. 2. Categories of patients by age group.

As shown in table 2, there is a significant statistic difference between obtained distribution and the theoretic one (uniform).

Table 2. One-Sample Chi-Square Test Summary.

Total N	120
Test Statistic	86.200[a]
Degree Of Freedom	5
Asymptotic Sig. (2-sided test)	.000

Note: There are 0 cells (0%) with expected values less than 5. The minimum expected value is 20.

Based on the results of our study, the biggest number of cases of pericoronitis was observed in the vertical position (92 cases) by Winter, followed by the mesioangular position (24 cases) and the distoangular position (4 cases), as shown in table 3. In the study, we did not detect any patients with a horizontal position.

According to the classification by Pell and Gregory shown in Table 4, most cases belonged to Class I (73 cases). Third molars classified in position A (92 cases) had a greater chance of pericoronitis when compared to those in position B (24 cases) or C (4 cases).

Table 3. *Wisdom tooth position by Winter classification.*

Tooth position by Winter classification	Vertical	Total	92
		Column N %	76.7%
		95.0% Lower CL for Column N %	68.5%
		95.0% Upper CL for Column N %	83.5%
	Mesioangular	Total	24
		Column N %	20.0%
		95.0% Lower CL for Column N %	13.6%
		95.0% Upper CL for Column N %	27.8%
	Distoangular	Total	4
		Column N %	3.3%
		95.0% Lower CL for Column N %	1.1%
		95.0% Upper CL for Column N %	7.7%
	Horizontal	Total	0
		Column N %	0.0%
		95.0% Lower CL for Column N %	.
		95.0% Upper CL for Column N %	.

Note: CL – confidence limit; N – number; Upper 95% confidence limit (CL) – sample mean + (1.96); Lower 95% CL – sample mean – (1.96).

Table 4. *Wisdom tooth position by Pell&Gregory classification.*

Tooth position by *Pell&Gregory* classification	I	Total	73
		Column N %	60.8%
		95.0% Lower CL for Column N %	51.9%
		95.0% Upper CL for Column N %	69.2%
	II	Total	42
		Column N %	35.0%
		95.0% Lower CL for Column N %	26.9%
		95.0% Upper CL for Column N %	43.8%
	III	Total	5
		Column N %	4.2%
		95.0% Lower CL for Column N %	1.6%
		95.0% Upper CL for Column N %	8.9%
	A	Total	92
		Column N %	76.7%
		95.0% Lower CL for Column N %	68.5%
		95.0% Upper CL for Column N %	83.5%
	B	Total	24
		Column N %	20.0%
		95.0% Lower CL for Column N %	13.6%
		95.0% Upper CL for Column N %	27.8%
	C	Total	4
		Column N %	3.3%
		95.0% Lower CL for Column N %	1.1%
		95.0% Upper CL for Column N %	7.7%

Note: CL – confidence limit; N – number; Upper 95% confidence limit (CL) – sample mean + (1.96); Lower 95% CL – sample mean – (1.96).

Discussions

Pericoronitis is an inflammation that occurs in the soft tissues around an erupting tooth. Due to the fact that pericoronitis is particularly related to the complications caused by the eruption of teeth, the third molars, especially the lower ones, are particularly more affected, since they present more limitations and difficulties in the complete eruption, mainly due to lack of space and bad dental positioning [5].

Patients with pericoronitis experience pain, discomfort, swelling, pus discharge, lymphadenopathy, dysphagia, systemic disorders, and serious complications [6].

Clinical studies suggested that the microbiota predominantly responsible for pericoronitis is mainly anaerobic and contains *Campylobacter* species, *Capnocytophaga* species, *Fusobacterium* species, *Micromonas (Peptostreptococcus) micros*, *Prevotella intermedia*, *Prevotella nigrescens*, and *Veillonella* species. Streptococci, staphylococci, actinomycetes, and enterobacteria, as well as protozoa and fungi, have also been described [7].

Based on the results of our study, we can affirm that pericoronitis is more frequently encountered in females as compared to males; this can be explained by the multiple theories from the specialized literature, such that as female`s jaws stop growing when the third molars just begin to erupt, in contrast to males, in whom the growth of the jaws continues beyond the time of the eruption of the third molars [8]. Our study shows that maximum number of cases of pericoronitis was observed in the age group 21–25 years (53 patients), followed by 26–30 years in 30 cases, and 31–35 years in 15 cases. These results are very similar to those of the studies by Shin et al. [9] that found that the age group 20–29 years exhibited a maximum number of pathoses associated with a lower third molar and longterm exposure to irritants from the oral cavity. The present study showed that the biggest number of cases of pericoronitis were observed in the vertical position according to Winter. Similar results were noticed in the study conducted by Galvão et al., and McArdle et al, which concluded that a vertically impacted molar is commonly associated with pericoronitis [5, 10]. According to the classification of Pell&Gregory, there is a greater chance of pericoronitis in the position A, in contrast to the results by Galvão et al., who observed that pericoronitis is common in B position. The common symptoms such as swelling, trismus, pain, and difficulty in swallowing, were mostly seen in patients.

Conclusions

Mandibular third molar impaction is a common pathology encountered in young age groups. Dividing the patients into 6 age groups, the maximum number of cases of pericoronitis was observed in the age group 21–25 years, followed by 26–30 years. According to Pell&Gregory's classification, the most common impaction position in pericoronitis occurrence is vertical, followed by the mesioangular position. According to another classification by Pell and Gregory, most cases belonged to class I, position A.

Authors' contributions

Gabriela Motelica – Conception and design of study; Gabriela Motelica – Analysis and interpretation of data.

Declaration of conflict of interests

There is no conflict of interest.

Financial disclosure statement

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REVIEW ARTICLE

Molecular and cellular biomarkers in status epilepticus and epilepsy

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Short title: *Molecular and cellular biomarkers in SE and epilepsy*

What is not yet known on the issue addressed in the submitted manuscript

Molecular and cellular biomarkers in combination with imaging and electrical investigations will provide a more specific platform for defining epileptogenic zone.

Research hypothesis

Analysis and selection of the most informative molecular and cellular biomarkers predictive of epileptogenesis.

The novelty added by manuscript to the already published scientific literature

Peripheral biomarkers have numerous uses in the treatment, prognosis, and pharmacovigilance of epilepsy. Epilepsy is represented by a research area of specific biomarkers, which are of great clinical importance, being necessary for forecasting the disease, the risk of developing neurological sequelae and pharmacoresistant to antiepileptic remedies. Thus, their identification could have a significant impact on the clinical course of the disease.

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Summary

Introduction. Peripheral biomarkers have numerous uses in the treatment, prognosis, and pharmacovigilance of epilepsy. Unfortunately, no peripheral biomarker has demonstrated proven efficacy, although several options are being investigated. In this article, we want to analyze the main areas in which peripheral biomarkers can present their usefulness, including participation in the processes of inflammation, dysfunction of the blood-brain barrier, changes in metabolism, hormones, and growth factors.

Material and methods. Publications on diagnostic biomarkers of epilepsy were reviewed. References were identified by PubMed, MEDLINE and Scopus search until June 2022, with various combinations of the terms – „epilepsy", „seizures", „epileptogenesis", „biomarkers", „neuroimaging", „inflammation", „status epilepticus", „prognosis". A qualitative and analytical study was performed focused on primary studies published in 2020-2022. More than 85 sources were identified and 33 were selected for analysis from the PubMed, MEDLINE, and Scopus online databases. 12 articles, 5 clinical trials, 2 meta-analyses, 7 reviews, and 7 systematic reviews were identified.

Results. Screening articles from online databases according to the search criteria, we found 258 titles on molecular and cellular biomarkers in epilepsy highlighted. The final bibliography included 33 sources that summarized that biomarkers of epileptogenesis are expensive and difficult to research, but the identification of biomarkers specific to the entire epileptogenic process, in close proximity to neuronal damage, have demonstrated the possibility of predicting the risk of seizures, epilepsy and resistance to treatment.

Conclusions. Epilepsy remains a continuous area of research; a special role is occupied by specific biomarkers of great clinical importance, being necessary for the prognosis of the disease, the risk of neurological sequelae, refractory to anti-epileptic drugs. Thus, their identification could have a significant impact on the clinical course of the disease.

Keywords: biomarkers, epilepsy.

Introduction

Epilepsy affects approximately 50 million people worldwide. One-third of them show drug resistance to antiepileptic drugs [1-3]. Specific biomarkers essential to determine drug resistance are not currently established. The biomarkers are defined as „cellular, biochemical or molecular changes, which can be measured in biological environments, such

as human tissues, cells or fluids" [4, 5]. More recently, a working group of the National Institutes of Health (NIH) extended this definition to „a characteristic that can be objectively measured and that is an indicator of normal and pathological biological processes or pharmacological response to applied therapeutic intervention" [6]. In the case of epilepsy, the spectrum of biomarkers ranges from neuroimaging and electrophysiological markers to molecular and cellular markers, determined in peripheral fluids and tissues.

This article is a review article on soluble biomarkers, determined in blood and cerebrospinal fluid (CSF). They may have multiple potential uses such as the ability to predict the development of epilepsy following a brain injury and/or after a first seizure, the progression of the disease and its severity, and the possibility of developing drug resistance to antiepileptic drugs [4]. Thus, bibliographic sources were analyzed in which data related to the importance of biomarkers in epilepsy were exposed.

It is known that the biomarkers of epileptogenesis are expensive and difficult to research. Even after severe brain conditions, in the presence of a potential epileptogenic risk, such as severe head trauma, the proportion of people who develop epilepsy is not high. Moreover, this process can take decades. An ideal situation would be to identify the biomarkers specific to the entire epileptogenic process, in close proximity to neuronal injury, with the possibility of predicting the risk of developing seizures, epilepsy, and resistance to treatment [7, 8].

Molecular and cellular biomarkers should ideally be present in an accessible compartment such as blood, tissues, CSF, sputum, or urine (Figure 1) [9].

Material and methods

Publications on diagnostic biomarkers of epilepsy were reviewed. References were identified by a search in the PubMed, MEDLINE and Scopus databases for articles published until June 2022, with various combinations of the terms – „epilepsy", „seizures", „epileptogenesis", „biomarkers", „neuroimaging", „inflammation", „status epilepticus", „prognosis". A qualitative and analytical study was performed focused on primary studies published between 2020-2022 and dedicated to the identification of biomarkers for diagnosis, disease progression, and complications of epilepsy. The articles contain a detailed analysis and a synthesis of recommendations regarding the targeted selection of biomarkers in the diagnosis of epilepsy. Articles were selected taking into consideration the title and their abstracts. More than 85 sources were identified and 33 were selected for analysis. 12 articles, 5 clinical trials, 2 meta-analyses, 7 reviews, and 7 systematic reviews were identified.

Results and discussion

Inflammation

Recent experimental studies have revealed that inflammation can precipitate seizures and support convulsive activity [10-12]. In addition, it can influence epileptic discharge by changes in glutamate and ions homeostasis involved in the epileptogenic process. Consequently, biological markers of inflammation represent a pathway for identifying patients in whom inflammation plays a key role in epileptogenesis and/or maintaining neuronal excitability [10, 11]. Furthermore, immunomodulatory drugs, including steroids and intravenous immunoglobulins, have been shown to have successful therapeutic effects in some children with epileptic encephalopathies, which are often resistant to conventional antiepileptic drugs (AED).

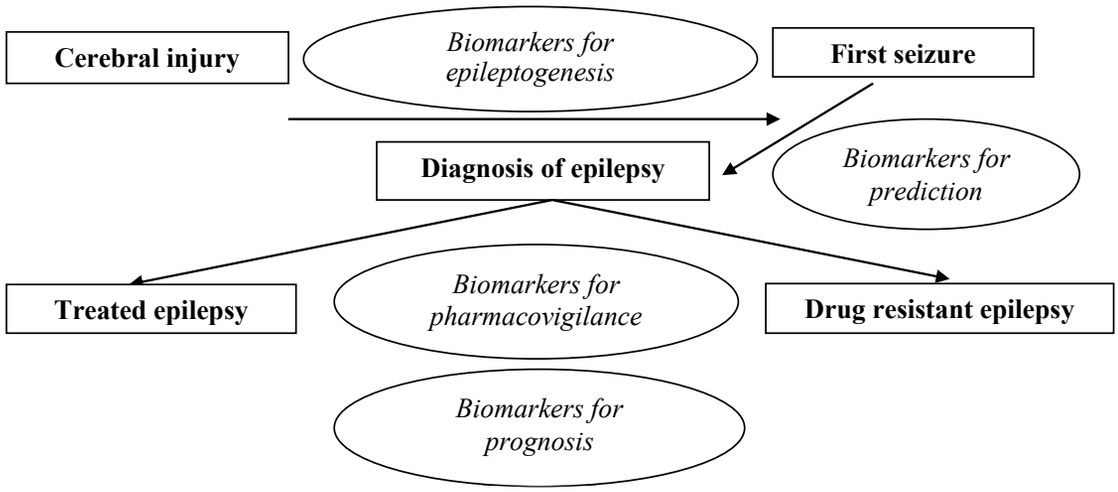

Fig. 1. Types of biomarkers involved in pathogenetic process of epilepsy.

Inflammation of the tissues can be involved not only in the generation of seizures but also in the development of the drug resistance phenotype. Surprisingly, even children with focal seizures, seizures that are not traditionally considered to be inflammatory in nature, have a positive response to steroids. Targeting the inflammatory process may

present a novel therapeutic strategy in the treatment of epilepsy and circulating biomarkers capable of demonstrating the response to treatment have an increased value [12, 13].

In several studies, individuals with drug-resistant focal epilepsy have been shown to exhibit an imbalance in the ratio of interleukin-1 / interleukin-1 receptor antagonists (IL-1/IL-1RA). IL-1 is recognized as a mediator of brain inflammation. In rodents, pharmacological blocking of IL-1 biosynthesis significantly reduces the risk of developing seizures. This "pro-inflammatory cytokine profile" in peripheral blood, consisting of elevated IL-6 levels with a low IL-1/IL-1RA ratio, may be found among patients in whom persistent and unsolved inflammation leads to the development of neuromodulation associated with changes in neuronal excitability. Likewise, a higher concentration of IL1-Elm in serum and CSF was associated with an increased risk of developing epilepsy after brain damage [14].

The high-mobility group box-1 (HMGB1) is one of the best-known mediators of neuroinflammation evoked by epileptogenic lesions, a fact proven in clinical models of epilepsy [15]. It is actively released by immune cells during injury-induced infection or aseptic inflammation. Cellular death leads to the passive release of HMGB1. Experimental models of epilepsy suggest that the acetylated disulphide form of HMGB1 is responsible for the occurrence of inflammation in epilepsy. A pilot study in patients with resistant focal epilepsy, suggests that HMGB1 isoforms can be considered candidate biomarkers in epilepsy stratification [16]. However, HMGB1 is not only specific for epilepsy and in fact, it is a sensitive and specific biomarker for several conditions, including autoimmune pathologies and malignancies.

Pharmacological inhibition of HMGB1 has been successful in numerous experimental disease models [17]. Interventions used included direct inhibition using polyclonal and monoclonal antibodies, competitive HMGB1 a-Box inhibitors, HMGB1 sequestration, and degradation methods. Since HMGB1 has no cerebral specificity, it is unclear whether peripheral or central nervous system (CNS) changes are responsible for seizures, or the therapeutic effects described above.

Blood-brain barrier

Dysfunction of the blood-brain barrier following prolonged seizures in animal models has been recognized since the 1950s. Vasogenic edema, caused by neurovascular changes, was first described by Klatzo and colleagues. In some cases, dysfunction of the blood-brain barrier can cause seizures, while dysfunction created artificially by other means leads to delayed epileptogenesis [18, 19]. Impaired blood-brain barrier function due to a hypertensive crisis in eclampsia and hypertensive encephalopathy may involve changes in serum magnesium levels. Injury to the blood-brain barrier in experimental studies caused the delayed occurrence of seizures [20, 21]. Impaired blood-brain barrier function is commonly found in various neurological conditions, such as encephalitis, meningitis, stroke, Alzheimer's disease, and other CNS disorders. There is no doubt that cerebrovascular dysfunction conditions or sustains seizures. The role of cerebrovascular impairment in various CNS disorders, including epilepsy, has been clinically accepted in the past, but only recently, it has been tested as an important mechanism underlying epileptogenesis. In the case of human epilepsy, the results of the studies are suggestive for a loss of selective permeability of the blood-brain barrier in the focal regions from which the convulsions originate [22, 23].

In addition, data from several studies support the idea that the blood-brain barrier in patients with epilepsy exhibits a variety of molecular changes that are in one way or another involved in epileptic disease [18-20]. Given the importance of the blood-brain barrier for convulsive disorders and epilepsy, it is not surprising that biomarkers in this pathophysiological field are highly examined, but at the same time, they require further research. In general, there are three approaches to determine the integrity of the blood-brain barrier in epilepsy. They do not differ from aspects previously used to measure cerebrovascular integrity in other neurological diseases [7]. Historically, the ratio of serum albumin to CSF albumin was the first used approach. It is known that the vascular barrier protects the brain from harmful substances in the blood while providing the necessary nutrients to the brain for proper functioning and strict regulation of the traffic of cells and molecules from the blood to the brain. The vascular barrier also separates impermeable macromolecules (>~500 Da) in the blood and brain. Thus, when the blood-brain barrier is intact, the albumin is 10 times more concentrated in the blood and will have a constant ratio to the concentration in the CSF [20]. A similar principle, but applied in another way, would be the nuclear magnetic resonance imaging (MRI) with the application of contrast agents. In this case, the „ratio" of the cerebral parenchyma to the blood is measured topographically, and the distribution of the substance injected into the blood is visualized in the brain. The absence of extravasation indicates an intact blood-brain barrier. Thus, the brain produces specific proteins that are isolated in the CNS in conditions of the intact blood-brain barrier. In there is a lesion of the blood-brain barrier, proteins, which are normally present in high concentrations in the CNS, are free to diffuse into the blood according to their concentration gradient. An ideal peripheral biomarker of clinical significance, should be: (1) a protein (or molecule) present in small or undetectable concentrations in the serum of healthy subjects; (2) present in the brain and CSF, being in a higher concentration in the cerebral parenchyma than in plasma; (3) available for extravasation in case of damage to the blood-brain barrier; and, (4) further released by brain cells in response to brain damage (for example, during reactive gliosis). Several proteins, including S100 calcium-binding protein B (S100B), neuronal-specific enolase (NSE), and glial fibrillary protein (GFAP) have been evaluated for their functions, and the S100B protein meets all the above-mentioned criteria. The imaging techniques available for clinical research do not have the high resolution required to „visualize" this structure, although the contrast agents that have been used to

measure the integrity of the blood-brain barrier. Functional assessment of the status of the blood-brain barrier by calculating the albumin coefficient (QA) in serum and CSF and MRI with contrast is widely accepted as the gold standard for determining the integrity of the blood-brain barrier. Recent work has shown that the serum level of the S100B protein correlates with QA, thus allowing the measurement of the CSF protein indirectly and without performing a lumbar puncture [21, 22].

It is relevant that serum S100B protein is the most studied marker, which may be involved in the mechanisms of delayed epileptogenesis after traumatic brain injury. Brain injury, in general, is associated with a rapid loss of integrity of the blood-brain barrier followed by the development of brain disease. Thus, the protein S100B emerged as a peripheral biomarker indicative of the permeability of the blood-brain barrier. The increase in serum levels of S100B reflects the presence of a damaged barrier and can predict long-term consequences after a brain pathology. Intracranial events are associated with an increased risk of seizures; thus, it is possible that the S100B protein will also prove to have a utility in detecting people at increased risk for seizures. Two independent meta-analyses for S100B in traumatic brain injury concluded that normal S100B levels accurately predict the results of a normal neuroimaging exam and that S100B sampling within 3 hours of injury should be considered when there is no focal neurological deficit or significant extracerebral injury [24, 25]. Therefore, there is an opportunity to conduct a test with a high predictive level, so that many unnecessary scans can be avoided, a test with a predictive value to diagnose complications after brain trauma.

CCL-2 is a pro-inflammatory chemokine produced by hyperactive neurons and microglia, it is involved in immune cell migration, chemoattraction of monocytic cells, disruption of blood-brain barrier and neuroinflammation. Its production is stimulated by IL-1β and tumor necrosis factor α (TNF-α) in the microglia, vascular endothelial cells, pericytes and neurons. The activation of CCL2 initiates the adhesion of monocytes to the inflamed endothelium, resulting in infiltration into the brain parenchyma [26, 27].

Aquaporin 4 (AQP4) is a membrane protein, serving as a water channel in conformity to the osmotic gradient; it is expressed by glial cells in the brain and the spinal cord. These channels are localized in the astrocytic plasma membrane that abut on brain micro vessels or on the pial covering of the brain surface. Both lowered expression and incorrect localization of AQP4 on end-foot membranes can lead to an alteration in the astrocytic function and homeostasis. The astrocytic AQP4 channels mediate water clearance in vasogenic edema with exacerbation of intracellular edema and so lead to cellular edema in the CNS. It was found that abnormalities in astrocytic ion channel expression, localization, and function could cause alteration of ion homeostasis and neurotransmitters, deposition of characteristic proteins, oxidative stress, and neuroinflammation. The overexpression and altered localization of AQP4 channels in astrocytes has been suggested as a reason for blood–brain barrier (BBB) dysfunction, manifested by the reduction in the flow of brain interstitial fluid mediated by AQP4 and due to a failure of clearance accumulation of pathologic markers such as amyloid-β (Aβ), and mutant α-synuclein (α-SYN) which trigger the production of reactive oxygen species (ROS) and inflammatory factors and contributes to the pathological progression of neurodegenerative diseases [28, 29].

Scientific evidence maintains the hypothesis that brain tissue inflammation is a central component for the development of seizures. Toll-like receptors (TLR) 4 is a trans-membrane protein that is highly expressed in the neurons, astrocytes, and microglia under pathological situations, such as cell injury and epileptic seizure [30, 31]. A study suggests a significant association between homozygous CC and heterozygous CT genotypes of TLR4 and epilepsy. It identifies these genotypes as a risk factor for epilepsy [32]. Some studies in rat models suggest that TLR4 signaling takes part in the drug-resistant epilepsy pathway. Inhibition of TLR4 downregulates IL-1β, TNF-α and NF-κB at the cellular level. The same study reveals that inflammatory pathways in drug-resistant epilepsy are activated. Such inflammatory factors as TLR4, IL-1β, TNF-α, NF-κB signaling factors and P-glycoprotein seem to be involved in the genesis of a drug-resistant epilepsy network. TLR4 was proposed to be an upstream regulator, that activates the downstream NF-κB, regulates inflammatory factors IL-1β, TNF-α, and other cytokines [33].

Conclusions

Epilepsy is represented by a research area of specific biomarkers, which are of great clinical importance, being necessary for forecasting the disease, the risk of developing neurological sequelae, and refractory to antiepileptic remedies. Thus, their identification could have a significant impact on the clinical course of the disease. It should be recognized that many of the biomarkers discussed in this review are also involved in other pathologies, including non-neurological diseases. Prospective research will be needed to identify individual or groups of specific biomarkers that would differentiate epilepsies from other diseases. New therapeutic strategies should involve integrating clinical information, including electroencephalography and neuroimaging exams, with new molecular and cellular biomarkers.

Identifying biomarkers of aberrant inflammation could stratify patients to determine who contributes to maintaining epileptic status. Focusing on inflammation, which plays a critical role in epilepsy may encourage the development of various strategies that halt the progression of the drug-resistant phenotype.

Markers of the integrity of the blood-brain barrier are useful tools for determining sequels in a variety of neurological diseases or acute events (strokes, trauma). Currently, the role of these markers in the prognosis and diagnosis of convulsive disorders is being studied, although, from, another point of view, these markers have already demonstrated that the blood-brain barrier is damaged now of

convulsive seizures and that the disruption of the integrity of this barrier is epileptogenic. Moreover, when the blood-brain barrier is damaged, allowing albumin to enter the interstitial space, can affect the effectiveness of some drugs and, therefore, markers of barrier integrity can be useful in therapeutic decision-making.

Better tools to predict the onset of epilepsy could lead to the development of new therapeutic strategies to prevent the development of epilepsy, potentially in the form of immunomodulatory intervention. In addition, early prediction of drug resistance would mean that patients could be evaluated for epilepsy surgery at an early stage, thus avoiding the administration of more antiepileptic drugs that are associated with side effects, are inevitably doomed to fail.

Declaration of conflict of interests

The authors have no conflict of interests to declare.

Authors' contribution

CC, IC, AC, LF, OC – the conception and the design of the study, the acquisition of data, the drafting of the manuscript; SH, SG – the critical revising of the manuscript for important intellectual content and the approval of the version of the manuscript to be published. All the authors – the analysis and the interpretation of data.

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REVIEW ARTICLE

Impact of vitamin D in chronic kidney disease and its effect on the musculoskeletal system

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What is not yet known on the issue addressed in the submitted manuscript

The role of vitamin D metabolism abnormalities in the pathogenesis of bone mineral disorders in chronic kidney disease is known, but it is not known the level of reduction of vitamin D in relation to the stage of nondialysis chronic kidney disease.

The research hypothesis

Chronic kidney disease is a global health problem, and a decrease in glomerular filtration rate increases the risk of bone mineral disorders and secondary hyperparathyroidism, which plays an important role in vitamin D deficiency.

The novelty added by manuscript to the already published scientific literature

Highlighting the decrease in vitamin D levels depending on the stage of chronic kidney disease.

Abstract

Introduction. Vitamin D plays an important role in maintaining musculoskeletal health. As the glomerular filtration rate decreases, vitamin D deficiency also occurs. The aim of this paper is to highlight the level of vitamin D depending on the stage of chronic kidney disease.

Materials and methods. A structured search was performed in the PubMed, Scopus and HINARI databases, where the relevant articles have been taken into account, published in the last 20 years. The search terms used (in English) were: „vitamin D deficiency", „pathogenesis of vitamin D", "the impact of vitamin D in chronic kidney disease", „chronic kidney disease".

Results. Several studies have shown that the change in vitamin D levels is dependent to the decrease of glomerular filtration rate. The lowest serum vitamin D concentration was observed in stage 5 of chronic kidney disease. Vitamin D deficiency occurs due to a decrease in the number of nephrons and a decrease in the number of proximal tubular cells that absorb vitamin D (25 (OH) D) to be subsequently hydroxylated to its active form by 1α-hydroxylase.

Conclusions. Patients with vitamin D-deficient due to chronic kidney disease have an increased risk of decreased bone mineral density and multiple fractures.

Keywords: vitamin D, deficiency, pathogenesis, chronic kidney disease

Introduction

Vitamin D plays an important role in maintaining musculoskeletal health. As the glomerular filtration rate decreases, vitamin D deficiency also occurs. This is due to the decrease of functional nephrons and in the number of proximal tubular cells that absorb vitamin D 25 (OH) D, which is then hydroxylated in its active form by 1α-hydroxylase. It is well known that the abnormalities of vitamin D are important factors in the pathogenesis of bone mineral disorders. At present there is more and more data about the role of vitamin D on muscle health and function [1-3]. It is known that patients with chronic kidney disease have clinical manifestations such as: muscle pain and weakness, sarcopenia, fatigue, low exercise tolerance, fractures and falls that may have an adverse effect on the quality of life [4-9, while vitamin D deficiency is directly related to the severity of muscle manifestations [10]. Multiple data show that the vitamin D receptor (VDR) is expressed in muscles and that the vitamin D receptor regulates gene expression and the absorption of 25 (OH) D in skeletal muscle cells [11, 12]. Vitamin D is part of a group of fat-soluble vitamins. There are two

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forms of vitamin D: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is the plant-derived form (ergosterol or provitamin D2). Vitamin D3 comes either from food of animal origin, or is synthesized in the skin from 7-dehydrocholesterol (provitamin D3) under the action of ultraviolet radiation [13]. Vitamin D activation takes place in two stages: the first stage takes place in the liver, and the second stage takes place in the kidneys. In plasma, vitamin D is transported as a complex with a specific alpha1 globulin-vitamin D transporter protein. The first hydroxylation takes place in the liver, where 25-OH vitamin D (calcifediol), a metabolite with limited biological activity, is formed. Then 25-OH vitamin D binds to a specific protein that is transported to the kidneys where the second hydroxylation takes place. Under the action of 1-alpha hydroxylase, the most active metabolite of vitamin D is formed: 1,25-(OH) 2 vitamin D (calcitriol) in the proximal renal tube.

Renal hydroxylation plays an important role in controlling the metabolism of vitamin D, which is regulated by serum calcium, phosphate and parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23), which is produced by bone osteocytes and osteoblasts [14].

The decrease in plasma concentration of both metabolites – 25 (OH) D and 1,25 (OH) 2D, is observed with the progression of chronic kidney disease [15]. There are known several factors contributing to vitamin D deficiency: renal dysfunction, dietary restrictions, reduced sun exposure, skin hyperpigmentation, diabetes, obesity, the presence of uremic syndrome, hyperphosphatemia, metabolic acidosis, proteinuria and increased FGF23 [16].

The role of vitamin D in the kidneys is to increase the tubular reabsorption of calcium. Thanks to the maintenance of calcium homeostasis, calcitriol has an important role in the process of bone remodeling. Along with the interaction of specific receptors, it induces the expression of bone matrix proteins (osteopontin, osteocalcin, alkaline phosphatase) and inhibits the synthesis of type I collagen. At the same time, there is an increase in bone resorption together with the action of parathyroid hormone, by stimulating immature osteoclastic precursors, which will later turn into mature osteoclasts. They remove calcium and phosphorus from the bone, maintaining the levels of calcium and phosphorus in the blood.

Normal concentrations of Ca^{2+} and phosphorus in the blood favor the mineralization of the osteoid. Vitamin D deficiency leads to disorders of osteoid mineralization, as a result favors the appearance of osteomalacia.

Materials and methods

A search of scientific papers published since 2001 in the MEDLINE electronic database was performed using the search engine PubMed, Scopus and HINARI (Health Internet Work Access to Research Initiative) - Research-4Life program. We have selected English articles provided by these platforms. The search terms used were: „vitamin D deficiency", „pathogenesis of vitamin D", „the impact of vitamin D in chronic kidney disease", „chronic kidney disease". Original articles, meta-analyzes and systematic reviews were selected.

Results and discussion

After processing the information from the PubMed, Scopus and HINARI databases, according to the search criteria, 220 articles on vitamin D deficiency in chronic kidney disease were selected. The final bibliography contains 38 relevant sources, which were considered representative of the material published on the topic of this synthesis article. Excluded from the list were the content of publications that did not reflect the research topic, as well as articles that were not accessible through the HINARI database.

Vitamin D deficiency in chronic kidney disease

The fibroblastic growth factor 23 (FGF23), which is increased in chronic kidney disease, inhibits the activity of 1α-hydroxylase, which subsequently stimulates 25-hydroxylase, and, at the same time, vitamin D further increases the production of FGF23 [14, 17]. Increased FGF23 stimulates increased renal phosphate excretion [18]. FGF23 inhibits alkaline phosphatase as a result. Moreover, FGF23 leads to extracellular increase in pyrophosphate, decreases the amount of inorganic phosphate, and stimulates the expression of the osteopontin gene.

Vitamin D 1,25 (OH) D binds to the vitamin D receptor (VDR), which is present in almost all tissues. The VDR-1,25 (OH) 2D complex subsequently binds to the retinoic X receptor (RXR) which controls the transcriptional activity of target genes. 1,25 (OH) 2D has an important role in maintaining calcium and phosphate homeostasis, stimulating intestinal absorption and bone resorption [19]. The 1,25 (OH) 2D-VDR-RXR complex increases the expression of the epithelial calcium channel in the cells of the small intestine. This allows more calcium to enter the cell, ensuring the necessary availability of calcium and phosphate for the proper mineralization of the newly formed bone matrix. Vitamin D enhances the expression of LRP5, which, together with sclerostin, Dkk1 and frizzled, forms the Wnt pathway, which is an important process in bone mineralization [20, 21]. In most patients with chronic kidney disease, 25-hydroxyvitamin D levels were observed to be <30 ng/ml, which is lower than normal. In patients who have a high level of proteinuria have an even lower level of vitamin D. Interestingly, there was a positive relationship between 25-hydroxyvitamin D levels and 1,25-dihydroxyvitamin D levels, in contrast to patients without chronic kidney disease.

Muscle damage in vitamin D deficiency

Several studies have shown that in addition to maintaining calcium homeostasis, which is imported for muscle function, vitamin D deficiency can act directly on skeletal muscle. Vitamin D also binds to the vitamin D receptor in muscle cells. This, in turn, leads to the rapid up-regulation of calcium channels [22]. The influence of vitamin D hypovitaminosis on skeletal muscle also refers to muscle structure. Structural muscle changes include disruption of the intermiofibrillar network, increased intramuscular lipids, and rapid atrophy of white fibers (type 2) [23-25]. Subjects

with severe vitamin D deficiency show generalized muscle atrophy, before the appearance of biochemical signs of bone disease [26].

Multiple studies show that the presence of myopathy in chronic kidney disease occurs at a GFR <25 mL/min/1.73m^2 and increases with worsening of renal function [27, 28]. The diagnosis of uremic myopathy is established based on clinical manifestations: weakness and sarcopenia found mainly in the lower limbs [29]. Meanwhile, muscle enzyme levels and electromyographic studies are usually normal [30]. These characteristics are also found in patients with vitamin D deficiency [23].

Bone damage in vitamin D deficiency

Skeletal disorders associated with bone mineral disorders in chronic kidney disease are associated with bone loss and fractures. Compared to the general population, the incidence rates of fractures are more than four times higher and are associated with higher morbidity and mortality. With the progression of chronic kidney disease, metabolic disorders risk is higher, including abnormal bone remodeling, which leads to to osteoporosis and subsequent decrease in bone strength. Chronic kidney disease is a risk factor for osteoporosis. Osteoporosis is a skeletal condition characterized by compromised bone density that increases the risk of fracture [31]. Bone mineral density measurement using osteodensitometry by Dual X-ray Absorption (DXA) is a commonly used method for assessing bone mineral density. In chronic kidney disease, the relation among bone mineral density, bone fragility and the risk of fracture is not always clear. In chronic kidney disease there is a greater loss of cortical bone than trabecular bone, due to the presence of hyperparathyroidism compared to postmenopausal osteoporosis, where there is loss of trabecular bone in the axial skeleton [32]. The preferred sites for measuring bone mineral density in patients with chronic kidney disease are the hip and radio-carpal joints. The use of computed tomography could also be a useful tool for assessing bone loss and micro-architectural changes [33]. Although there have been doubts about the usefulness of bone mineral density in chronic kidney disease [34], measuring bone mineral density may become useful in these patients. This issue is currently being reviewed and bone mineral density testing is most likely to be recommended in patients with chronic kidney disease and evidence of bone mineral disorders or in patients with CKD who have risk factors for osteoporosis, especially if the results may change the management [35, 36].

Bone biopsy is not commonly performed in uremic patients, although it is the gold standard and the only way to assess the type of renal osteodystrophy in bone mineral disorders in chronic kidney disease [37, 38]. Bone histological changes in chronic kidney disease range from low bone turnover, mineralization disorders, and changes in bone volume. These histological changes may occur alone or together. The prevalence of renal osteodystrophy in bone mineral disorders in chronic kidney disease is high; the presence of osteoporosis is usually a diagnosis of exclusion.

Conclusions

Vitamin D participates in the control of bone metabolism and calcium homeostasis and plays an important role in muscle function in chronic kidney disease. Patients with chronic kidney disease with vitamin D deficiency have a high risk of decreased bone mineral density and multiple fractures due to mineralization defects. The clinical manifestations observed in patients with chronic kidney disease correlate with levels of 25 (OH) D.

Conflict of interests

Nothing to declare.

Authors' contribution

All authors contributed equally to the research, data analysis, and writing of the manuscript. All authors read and approved the final article.

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REVIEW ARTICLE

Recovery of patients with gout

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What is not yet known on the issue addressed in the submitted manuscript

Complex recovery within the framework of dietary, pharmacological recommendations for patients with gout.

Research hypothesis

A literature review was conducted to generalize information about dietary and pharmacological recommendations for patients with gout.

The novelty added by manuscript to the already published scientific literature

For the first time, recommendations on dietary, drug treatment for patients with gout are summarized and systematized.

Abstract

Introduction. The incidence and prevalence of gout have increased worldwide in recent decades. Scientists at the Rochester Epidemiology Project (MN, USA) have seen a two-fold increase in the incidence of primary gout (patients without diuretic exposure) over a 20-year period, which ended in 1996. The increase of incidence may be related due to the difficulty and often unsatisfactory treatment options. The aim of the study was to systematize the recommendations on dietary treatment, and medication for patients with gout.

Materials and methods. An analytical, qualitative, and secondary study was performed in the form of a synthesis article. 115 sources were identified and analyzed; from this list, 44 sources were selected according to the impact score during the publication period and according to the level of recommendations.

Results. 44 articles were included. Most studies were small, retrospective analyses performed in single centers, with concerns for bias. Eleven studies (including five randomized controlled trials) reported improved patient outcomes following pharmacological interventions with known efficacy in gout, including allopurinol, prednisolone, NSAIDs and anakinra. Eight studies reported improved outcomes associated with non-pharmacological interventions: inpatient rheumatology consultation and a hospital gout management protocol. No studies to date have prospectively evaluated strategies designed to prevent re-admissions of patients hospitalized for gout flares.

Conclusions. Urate crystals is completely soluble when we can lower the serum level of uric acid to normal values, but this often requires long-term treatment. The early onset of rehabilitation of affected joints helps to reduce the articular inflammatory process, the pain syndrome and it delays the progression of the underlying pathology while improving the quality of life in patients with gout. Further research is needed to enable healthcare providers to individualize and optimize gout treatment strategies, ensuring that patients with gout receive effective, safe, and high-quality care.

Keywords: gout, recovery, management, prevention.

Introduction

Gout is a common metabolic disease manifested by recurrent inflammatory arthritis, which impairs patients' quality of life [1, 2]. In addition, gout and hyperuricemia increase the risk of associated cardiovascular complications and shorten patients' life expectancy [3-6].

Recent ACR guidelines have adopted a treatment strategy focused on sustained reduction of sodium urate crystals

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deposition in tissues [7-9] and long-term maintenance of low plasma urate levels (<6 mg/dL (<0.36 mmol/L)).

Patients with severe gout (presence of tophi, chronic arthropathy, and frequent relapses) are recommended to maintain even lower plasma sodium urate levels (<5 mg/dL (<0.30 mmol/L)). According to the 2012 EULAR guidelines, there is a consensus that urate-reducing drugs should be only used in patients with established gout [10-12].

The aim of the study

To evaluate the dietary and pharmacological recovery options in patients with gout.

Materials and methods

We searched for articles from 2012 to June 2022, using the terms „gout", „diet", „drugs", „topical treatment" and their synonyms in the following databases: PubMed, Cochrane Library, EMBASE, International Pharmaceutical Abstracts, American College of Rheumatology (ACR), European Alliance of Rheumatology Associations (EULAR).

The topics of the papers that we reviewed to write our article were related to the evaluation of the effectiveness of the treatment process (medication, diet therapy, physical therapy) for gout (in remission and exacerbation stages).

Results and discussions

After analysis of the search results, 44 publications were selected, which included randomized controlled trials, examinations and treatment protocols for patients with gout.

Treatment efficacy was determined against the background of registered drugs (allopurinol, febuxostat, glucocorticoids, NSAIDs, and biological therapy). The effectiveness of inpatient and outpatient treatment was evaluated, but unfortunately, to date, there is no data on the rehospitalization of these patients and the effectiveness of the outpatient treatment.

Currently, in addition to the „gold standard" in which monosodium urate crystals (MUC) in synovial fluid or tophus aspirate are identified, there are also methods of non-invasive examination. In 2015, the OMERACT working group proposed guidelines that describe all methods for the diagnosis of gout, which include invasive and non-invasive methods of examination. The main principles in evaluating gout treatment are recommended to consider the dynamics of urate deposition, joint inflammation, and bone erosion [42, 43].

Clinical parameters of monitoring during therapy can be Xray to determine the size of the tophi; ultrasonography, which demonstrates the presence of double contour sign and dual-energy computed tomography (DECT which determines the composition of various tissues as well as helps to detect crystal accumulations in the area of inflammation and allows visualization of the musculoskeletal system) can also be important indicator, which was confirmed by ACR/EULAR systematic literature review on gout imaging [2, 5, 8, 12-16, 27, 38, 42].

The main treatment for both acute and chronic forms of gout are well-known drugs [22-27].

Glucocorticoids. Oral glucocorticoids are often used in patients with a typical gout flare who can take oral medications but have contraindications for nonsteroidal anti-inflammatory drugs [23-25]. The dosing regimen of glucocorticoids is chosen individually for the patient depending on the severity of the flare (duration, dose, and routes of administration). Glucocorticoids in a short course of treatment show high effectiveness and have less risk of side effects compared to other drugs used to treat acute gout. Intra-articular injection of glucocorticoids may be recommended for those who cannot take oral medications. In addition, parenteral glucocorticoids may be indicated among those who cannot take medication orally and are not candidates for intra-articular therapy (e.g., for active inflamed joints >2). Intravenous methylprednisolone (20 mg) may be useful among those with polyarticular involvement, with intravenous access, and without contraindications to glucocorticoids. Intramuscular treatment with triamcinolone acetate (40-60 mg) may be an alternative treatment for patients with similar conditions [23-25].

Nonsteroidal anti-inflammatory drugs (NSAIDs) are very good alternatives to oral glucocorticoids in the treatment of acute gout [24-26]. They are particularly appropriate in the younger patients who do not have a renal, cardiovascular, or active gastrointestinal disease. Naproxen (500 mg twice daily) or indomethacin (50 mg three times daily) are usually used. However, other NSAIDs such as ibuprofen (800 mg three times a day), diclofenac (50 mg twice three times a day), celecoxib (100 mg twice a day), and meloxicam (15 mg a day) are just as effective. The effectiveness of NSAIDs is best seen within the first 48 h of a flare of gout and can be discontinued two to three days after clinical symptoms disappear. However, there are contraindications to the use of NSAIDs: chronic kidney disease (with creatinine clearance <60 ml/min), active gastrointestinal ulcers, cardiovascular disease (especially heart failure), or concomitant treatment with anticoagulants. Side effects from short-term use of NSAIDs are rare but include gastrointestinal distress and impaired renal function [26]. Triamcinolone acetonide (up to 40 mg for large joints and 20 mg for medium joints) or methylprednisolone acetate is commonly used. Although the evidence for its use in the treatment of gout flares is limited, it can be a relatively safe and effective treatment choice.

The prescription of *colchicine* is most often associated with the ineffective use of NSAIDs. Colchicine in doses of 0.5 - 1.0 mg (maximum dose of 2 tablets), with long-term use, has no side effects (including cardiovascular complications) in 90% of patients, which has been proven in numerous randomized trials [27, 28, 32, 33, 40].

Interleukin-1 (IL-1) inhibitors. Although IL-1 inhibitors may be beneficial for some patients with acute gout attacks, they are usually reserved for those for whom other available treatments have failed or for those with contraindications [29]. Anakinra (100 mg daily) is the preferred IL-1 inhibitor for the treatment of acute gout because of its short half-life and relatively modest cost compared with other IL inhibitors. It is administered subcutaneously daily until gout exacerbation symptoms subside and may be useful among patients with an active infection [30].

Allopurinol according to the international guideline is the drug of choice at the beginning of gout treatment [34]. Its dose varies from 100 mg to 800 mg per day. Most often,

patients take a maintenance dose of 100 mg and a treatment dose of 300 mg per day. If gout is resistant, the dosage can increase, but so does the frequency of side effects, which include skin lesions (rash), kidney impairment (acute kidney injury), intestinal problems (diarrhea) and vascular system disorders (eosinophilia, thrombocytopenia) [20, 34, 35].

Febuxostat, like allopurinol, is a xanthine oxidase inhibitor and according to the latest recommendations is prescribed to patients with hyperuricemia and gout who cannot tolerate allopurinol [21, 36]. The daily dosage of febuxostat varies from 40 to 120 mg per day, preferably prescribed in the evening after meals. To prevent flares of gout during the first weeks of treatment, this drug is prescribed in combination with colchicine or NSAIDs. Side effects develop are less frequent compared allopurinol and efficacy is much higher. However, the following side effects have been described: increased transaminases and allergic manifestations [36-38].

Probenecid is the drug of choice in patients with gout who have impaired excretion of uric acid through kidney. Probenecid improves excretion, but in renal impairment, this drug is limited because it can worsen the renal function [39].

In addition to drug therapy, there are now dietary and lifestyle recommendations by the American College of Rheumatology and EULAR aimed at preventing metabolic disorders and reducing serum urate levels. The guidelines recommend a balanced diet rich in vegetables with adequate amounts of plant and animal foods and lifestyle modifications for patients with gout. The American College of Rheumatology has created multiple nonpharmacological dietary recommendations. These recommendations include general advices regarding diet and lifestyle modifications [13, 18, 41].

The 2021 EULAR guideline for lifestyle improvement in people with gout described nutritional supplements that can be included in patients' diets. New methods of dietary modification in gout patients are constantly being sought, particularly the use of supplements and vitamin C, but this has not proven to improve the quality of life of gout patients. The evidence for dietary impact on gout has been rated as low or very low [15-17, 19].

Lifestyle changes that include losing weight, stopping excessive alcohol consumption, and stopping purine-rich foods, reduce urate levels in patients with gout. Nevertheless, sometimes this is not enough, and patients are recommended to initiate pharmacological therapy. In gout, clear indications have been developed for the initiation of pharmacological therapy to reduce urate: frequent (2 yearly) flares of gout; the presence of a chronic form and the presence of a tophaceous form of gout [31, 41].

Conclusions

(1) Crystal urate deposition is fully reversible in cases where we can lower the serum level of uric acid to normal values, often requiring long-term treatment.

(2) The early rehabilitation of affected joints helps to reduce more effectively the articular inflammatory process, the pain syndrome, and it delays the progression of the underlying pathology and improves the quality of life of patients with gout.

(3) Further research is needed to enable healthcare providers to individualize and optimize gout treatment strategies, ensuring that patients with gout receive effective, safe and high-quality care.

Declaration of conflicting interests

Nothing to declare.

Authors' contribution

All authors contributed equally to the research, data analysis, and writing of the manuscript. All authors read and approved the final article.

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- ☐ au fost declarate orice potenţiale conflicte de interes.

Prin prezenta, autorii sunt de acord să transfere drepturile de proprietate (copyright) Revistei de Ştiinţe ale Sănătăţii din Moldova – Moldovan Journal of Health Sciences, în caz că manuscrisul va fi publicat.

Autorii (nume, prenume complet, semnătură):

1. _____
2. _____
3. _____
4. _____
5. _____
6. _____
7. _____
8. _____

Locul şi data: _____

Datele de contact ale autorului corespondent:

Instituţia: _____
_____

Adresa: _____
_____
_____

e-mail: _____
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Vă rugăm să transmiteţi acest formular completat şi scanat pe adresa: editor.mjhs@usmf.md

MJHS Moldovan Journal of Health Sciences
REVISTA DE ȘTIINȚE ALE SĂNĂTĂȚII DIN MOLDOVA

COVER LETTER

Manuscript title: _____
_____

Relevance of the manuscript for the Journal purposes: _____
_____
_____
_____

Contributions of the manuscript for to the research field: _____
_____
_____
_____

What is the added value of the manuscript to the already published scientific literature: _____
_____
_____
_____

We, the undersigned authors of the manuscript, declare that (please, tick):

- ☐ the paper contain original data;
- ☐ the paper has not been published before;
- ☐ the manuscript is not submitted for publication to another journal;
- ☐ all authors have contributed to the manuscript;
- ☐ the informed consent were obtained from all study subjects;
- ☐ all coauthors approved the final version of the manuscript;
- ☐ we agree for checking of the manuscript for plagiarism;
- ☐ any potential conflicts of interest were disclosed.

With this, the authors agree to transfer property rights (copyright) to the *Moldovan Journal of Health Sciences – Revista de Stiinte ale Sanatatii din Moldova*, in the event that the manuscript will be published.

Authors (name, surname, signature):

1. _____
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3. _____
4. _____
5. _____
6. _____
7. _____
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Revista de Ştiinţe ale Sănătăţii din Moldova

Moldovan Journal of Health Sciences

Ghidul autorului

Criterii pentru publicare

Articolele originale trebuie să conţină cercetări noi (originale), rezultatele cărora contribuie la acumularea de noi cunoştinţe în domeniul publicat şi cu condiţia că rezultatele prezentate nu au mai fost publicate înainte sau nu sunt depuse, în paralel, la o altă revistă, în vederea publicării.

Manuscrisele prezentate trebuie să corespundă standardelor STROBE (http://www.strobe-statement.org).

Prezentarea manuscrisului

Manuscrisele trebuie să fie prezentate doar în formă electronică, în limba română sau engleză. Dacă manuscrisul a fost depus doar în limba română, odată ce a trecut procedura de recenzare internă, acesta va fi tradus integral de către autori în limba engleză, pentru a putea trece procedura de recenzare externă. În acelaşi manuscris se permite utilizarea *US English* sau *British English*, dar nu mixt. Varianta tipărită (hârtie) nu va fi acceptată. Doar autorul corespondent va putea depune manuscrisul la redacţie; tot el va deţine responsabilitatea completă de procesul de depunere, de corespondenţa cu redacţia pe durata procesului de publicare.

Procesul de publicare poate fi amânat, întrerupt sau anulat, la discreţia autorului corespondent. Odată manuscrisul depus, autorul corespondent va primi un cod electronic de identificare a manuscrisului, pe care îl va folosi în corespondenţa ulterioară cu redacţia.

În scopul menţinerii integrităţii editoriale şi a standardelor internaţionale de calitate, Redacţia *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* utilizează un sistem de detectare a plagiatului şi va supune manuscrisul unei verificări antiplagiat. Depunerea manuscrisului pentru publicare înseamnă, implicit, acordul tuturor autorilor cu verificarea lui antiplagiat. În cazul suspectării că manuscrisul depus a încălcat politicile de publicare, acesta poate fi suspendat sau respins, indiferent de etapa procesului de publicare.

Scrisoarea de însoţire

La depunere, autorul corespondent va anexa la manuscris o scrisoare de însoţire. Formularul tipizat al Scrisorii de însoţire este oferit de către Redacţie. Scrisoarea de însoţire include: (1) titlul manuscrisului; (2) o scurtă descriere despre relevanţa manuscrisului pentru scopul promovat de Revistă; (3) contribuţiile aduse de manuscris pentru domeniul său; (4) modul în care manuscrisul adaugă valoare la literatura ştiinţifică de specialitate; (5) numele şi semnăturile tuturor coautorilor; (5) datele complete de contact ale Autorului corespondent, cu menţionarea instituţiei şi adresei instituţionale, nr. de telefon, nr. de fax şi adresa e-mail.

Instructions for Authors

Criteria for publication

Original articles should contain new (original) results, which bring new knowledge in the field. The submitted manuscripts should contain data unpublished before and not submitted in parallel for publication to another journal.

Manuscripts submitted must meet STROBE standards (http://www.strobe-statement.org).

Manuscript submission

Manuscripts must be submitted only in electronic form in Romanian or English. Once past the internal reviewing procedure, the manuscript was submitted only in Romanian will be fully translated by the authors in English to pass the external reviewing procedure. In the manuscript are allowed to use U.S. English or British English, but not mixed. Printed version (paper) will not be accepted. Only the corresponding author may submit the manuscript. The corresponding author holds full responsibility of the submission and correspondence with the editor during reviewing and publication process.

The publication of the manuscript may be postponed, stopped or canceled at the request of the corresponding author. Once the manuscript is submitted, the corresponding author will receive an electronic identification code of the manuscript, which should be used for subsequent correspondence with the editor.

In order to maintain editorial integrity and international quality standards, editor of the Moldovan Journal of Health Sciences reserves the right to use a plagiarism detection system. Thus the submitted manuscript will be checked for plagiarism. Manuscript submission involves agreement of all coauthors for checking for plagiarism. If the submitted manuscript violates copyright policies; it can be suspended or dismissed, regardless of the stage of the publishing process.

Cover letter

A submitted manuscript should be accompanied by a Cover letter. A template of Cover letter is provided by editor. Cover letter should include: (1) the title of the manuscript; (2) a short statement regarding the relevance of the manuscript for the journal proposes; (3) contributions of the manuscript for to field; (4) what is the added value of the manuscript to the already published scientific literature; (5) the names and signatures of all coauthors; (5) the full contact details of corresponding author, indicating the institution and institutional address, no. telephone, no. fax and e-mail.

In the Cover letter, the corresponding author should clearly indicate that: (1) the paper contain original data; (2) the paper has

În scrisoarea de intenţie, Autorul corespondent trebuie să indice în mod clar că: (1) lucrarea menţionată este originală; (2) lucrarea menţionată nu a fost publicată anterior; (3) lucrarea menţionată nu este depusă pentru publicaţie în altă revistă; (4) toţi autorii subsemnaţi au contribuit la elaborarea manuscrisului; (5) de la subiecţii incluși în studiu a fost obţinut consimţământul informat; (6) toţi autorii subsemnaţi au aprobat versiunea finală a manuscrisului; (7) acordul implicit de verificare antiplagiat al manuscrisului; (8) au fost declarate orice potenţiale conflicte de interes. De asemenea, Autorul corespondent poate include orice informaţie suplimentară în Scrisoarea de intenţie, dacă consideră că aceasta poate fi utilă pentru Redacţie.

Consimţământul informat

Orice manuscris care comunică rezultate experimentale, obţinute de la subiecţi umani, trebuie să fie bazat pe studii, în care a fost obţinut consimţământul informat de la subiect (ţi) şi/sau tutore (i). În scrisoarea de intenţie, autorul corespondent trebuie să indice în mod clar obţinerea consimţământului informat. În caz de necesitate, Redacţia este în drept să solicite probe suplimentare, care atestă obţinerea consimţământului informat.

Comitetul de Etică

Pentru orice studiu experimental, efectuat pe oameni sau animale, este necesar de a menţiona evaluarea etică a proiectului de cercetare. În acest sens, în articol vor fi menţionate numărul procesului verbal şi data şedinţei Comitetului de Etică, când a fost aprobat proiectul de cercetare.

Permisiuni

În conformitate cu ghidurile Comitetului Internaţional al Editorilor Revistelor Medicale (*ICMJE Guidelines*), în cazul când în manuscrisul prezentat este folosită sau reprodusă o informaţie publicată anterior, sau un material cu drepturi de autor, este de responsabilitatea Autorului corespondent să obţină permisiunea în scris a deţinătorului de drepturi (*Copyright*) şi să citeze corect sursa originală. Cu scopul de a menţine transparenţa, se recomandă ca această permisiune, sub formă de copie, să fie depusă împreună cu manuscrisul.

Fotografii cu pacienţi identificabili

În conformitate cu ghidurile internaţionale ale Comitetului de Etică a Publicaţiilor (*COPE Guidelines*), în cazul când în imaginile prezente în manuscris (fotografii, radiograme, rezultate de laborator, rezultatele investigaţiilor paraclinice, înregistrări video sau sonore ş. a.) o persoană este identificabilă fizic, de la aceasta trebuie obţinută o permisiune în scris de utilizare a imaginii date. Se recomandă ca permisiunea dată să fie depusă împreună cu manuscrisul, iar în manuscris să fie stipulat în mod clar, că această permisiune a fost obţinută.

Specificarea medicamentelor şi dispozitivelor

În manuscris se vor utiliza nume generice de medicamente, urmate, dacă este cazul, de denumirea lor comercială între paranteze. Pentru medicamente şi dispozitive, includeţi numele producătorului şi localizarea acestuia (ţara de origine).

Formatul fişierelor

Se acceptă următoarele formate de text pentru manuscrisul principal: Microsoft Word (97, 2003, 2007, 2010) şi formatele „.rtf“, „.doc“, „.docx“. Se acceptă următoarele formate pentru imag-

not been published before; (3) the manuscript is not submitted for publication to another journal; (4) all authors have contributed to the manuscript; (5) the informed consent were obtained from all study subjects (6) all coauthors approved the final version of the manuscript; (7) agreement for checking of the manuscript for plagiarism; (8) any potential conflicts of interest were disclosed. Corresponding author may include in the Cover letter any other additional information which could be useful for the editor.

Informed consent

Manuscripts that report experimental results obtained on human subjects must be based on studies in which informed consent was obtained from study subjects and/or their legal representative. The corresponding author should clearly indicate in his letter of intention about the obtaining of the informed. Editor reserved the right to request additional evidence attesting the obtaining of the informed consent.

Ethic Committee

For any experimental study conducted on humans or animals, it is necessary to mention in the article the ethical evaluation of the research project (such as date of evaluation and reference number of approval).

Permissions

In accordance with the guidelines of the International Committee of Medical Journals Editors (ICMJE Guidelines) if the submitted manuscript used or reproduced information/material previously published or copyrighted is the responsibility of the corresponding author to obtain a written permission from the owner of the copyright and properly cite the original source. In order to maintain transparency, it is recommended to submit the permission, as a copy, along with the manuscript.

Pictures

In accordance with international guidelines of the Publications Committee of Ethics (COPE Guidelines), if the manuscript contains pictures (photographs, radiograms, laboratory results, results of laboratory investigations, videos or sound etc.) which allows physical identification of the person, it must be obtained a written permission for the use of the image data. It is recommended to submit the permission along with the manuscript. Also in the manuscript text should be clearly stated that permission was obtained.

Drugs and devices specifications

In manuscript generic names of drugs, followed by their trade name in parentheses (if appropriate) should be used. For drugs and devices, manufacturer's name and location (country of origin) should be mentioned.

Files format

The following file formats for manuscript text are accepted: Microsoft Word (97, 2003, 2007, 2010) ".rtf", ".doc", ".docx". Pictures should be submitted in one of the following formats: ".jpeg", ". tiff", ".eps ", ".ppt", , ".pptx". The images could be transmitted also, in a format item ".ppt" or ".pptx" (one image one slide). Scanning

ini: „.jpeg“, „.tiff“, „.eps“, „.ppt“. „.pptx“. Este posibil ca imaginile articolului să fie transmise în format „.ppt“ sau „.pptx“ (o imagine – un slide). Calitatea imaginilor, indiferent de format, trebuie să fie, minim: pentru desene – 800 dpi, pentru imagini cu detalii fine – 1000 dpi, pentru imagini alb-negru – de 300 dpi.

Structura manuscrisului

Publicaţia Periodică *Moldovan Journal of Health Sciences – Revista de Ştiinţe ale Sănătăţii din Moldova* respectă recomandările STROBE de raportare a cercetărilor observaţionale biomedicale. Pentru a vă uşura procesul de elaborare şi structurare a manuscrisului, vă recomandăm să consultaţi informaţia respectivă, disponibilă online, pe site-ul www. strobe-statement.org.

Volumul textului unui manuscris nu trebuie să depăşească 6000 de cuvinte. Cu toate că numărul figurilor şi tabelelor în manuscris rămâne la discreţia autorilor, se recomandă ca numărul lor să fie limitate la 5, pentru a nu reduce din lizibilitatea articolului pe paginile Revistei.

Structura unui articol original trebuie să respecte următoarea consecutivitate:

- Titlul lung (formulat în conformitate cu ghidurile STROBE)
- Numele şi prenumele complete ale autorului (autorilor)
- Afilierile autorului (autorilor)
- Datele de contact ale autorului corespondent
- Titlul scurt (va fi utilizat în calitate de colontitlu pe paginile Revistei)
- Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).
- Rezumatul articolului (compus din: introducere, materiale şi metode, rezultate, concluzii), limitat la maximum 350 de cuvinte.
- Cuvinte cheie
- Introducere
- Materiale şi metode
- Rezultate
- Discuţii
- Concluzii
- Lista abrevierilor utilizate (dacă este cazul)
- Declaraţia de conflict de interese
- Contribuţiile autorilor
- Mulţumiri şi finanţare (dacă este cazul)
- Referinţe bibliografice
- Tabele şi legende la tabele (dacă este cazul)
- Ilustraţii şi figuri (dacă este cazul)
- Legendele figurilor (dacă este cazul)
- Descrierea datelor suplimentare, anexe (dacă este cazul)

Pe pagina de titlu a manuscrisului trebuie să fie prezente următoarele elemente:

- **Titlul manuscrisului:** formulat în conformitate cu ghidurile STROBE, trebuie să fie laconic, relevant pentru conţinutul manuscrisului, să reflecte tipul (*design*-ul) studiului şi să nu depăşească 25 de cuvinte. Nu se admit prezenţa abrevierilor în titlu.
- **Titlul scurt** (ce va fi utilizat drept colontitlu pe paginile Revistei) reprezintă o versiune scurtă, de esenţă, a titlului complet.

resolution should be as follows: drawings – at least 800 dpi, fine line images – 1000 dpi and greyscale images – at least 300 dpi.

Structure of the manuscript

Moldovan Journal of Health Sciences follows STROBE recommendations for reporting observational biomedical research studies. To facilitate the development of the manuscript, please consult this information available online at www.strobe-statement.org.

The volume of the manuscript text should not exceed 6000 words.

Although, the number of figures and tables in the manuscript is at the discretion of the authors, in order to not reduce article legibility it is recommended to limit their number to five.

Structure of original article must comply with the following sequence:

- Full title (according to the STROBE guidelines)
- Full authors' name
- Authors' affiliations
- Contact details of corresponding author
- Short title (to be used as a running head on the journal)
- Article highlights:
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already publishedscientific literature (limited to 1-3 sentences).
- Abstract (consisting of background, materials and methods, results and conclusions), to not exceed 350 words.
- Keywords
- Introduction
- Materials and methods
- Results
- Discussions
- Conclusions
- List of abbreviations used (if applicable)
- Declaration of conflict of interests
- Authors' contributions
- Acknowledgements and funding (if applicable)
- References
- Tables and tables' captions (if applicable)
- Pictures and figures (if applicable)
- Figures' legends (if applicable)
- Description of additional data, appendices (if applicable)

The cover page of the manuscript should include:

- **Title of the manuscript:** written according to the STROBE guidelines, should be concise, relevant to the content of the manuscript, and reflect the study design. The title length should not exceed 25 words. It is not allowed the presence of abbreviations in the title.

- **Short title:** (to be used as a running title) is a short version of the essential of the full title. Short title will be limited to 40 characters, including spaces.

- **Author(s) name:** Authors list must include only those persons who had a substantial contribution to the work. Exam-

Va fi limitat la 40 de caractere, inclusiv spaţiile.

• **Numele autorului (autorilor).** Autori sunt numiţi doar acele persoane, care au avut o contribuţie substanţială la lucrare. Exemple de contribuţie esenţială la lucrare sunt: elaborarea *design*-ului studiului, recrutarea pacienţilor, participarea în colectarea datelor, analiza datelor, interpretarea rezultatelor, scrierea propriu-zisă a articolului, realizarea tehnică a testelor, investigaţiilor, realizarea imaginilor, formularea concluziilor. Pot fi citaţi până la 10 autori individuali. În cazul când grupul de lucru depăşeşte 10 autori individuali, vor fi citaţi în secţiunea „Numele şi prenumele autorilor" doar primii doi, iar restul vor fi menţionaţi la sfârşitul articolului, la secţiunea „Mulţumiri şi finanţare".

Membrii grupului de lucru, care nu îndeplinesc criteriile formale de autor enumerate, dar au avut o oarecare contribuţie la lucrare, pot fi menţionaţi în secţiunea „Mulţumiri şi finanţare".

Notă: Pentru a diferenţia autorul corespondent şi autorii care au contribuit în aceeaşi măsură la lucrare, folosiţi caractere speciale, ca exponenţi, la sfârşitul numelor lor:

(*) – pentru Autorul corespondent;

(†) – pentru Autorii care au avut o contribuţie egală. (De exemplu: Adrian Belîi*, Adrian Belîi†)

Nu se vor menţiona gradele şi titlurile ştiinţifice şi cele ştiinţifico-didactice.

• **Afilieri.** Afilierea autorilor se va scrie după secţiunea „Numele autorului (autorilor)". În acest sens, se va menţiona numele complet al instituţiei de afiliere a autorului (autorilor), localitatea şi ţara.

Afilierea se marchează cu cifre arabe, în superscript (de exemplu: Adrian Belîi[1])

• Elementele scoase în evidenţă din articol:
  - Ce nu este, deocamdată, cunoscut la subiectul abordat (descris în 1-3 fraze)
  - Ipoteza de cercetare (formulată în 1-2 fraze)
  - Noutatea adusă de articol literaturii ştiinţifice din domeniu (limitată la 1-3 fraze).

Din pagină nouă:

Rezumatul

Rezumatul trebuie să fie scris la timpul trecut, persoana a treia. Acesta trebuie să ofere un sumar concis al scopului, obiectivelor, rezultatelor semnificative şi concluziilor studiului, în limitele la 350 de cuvinte, organizate în următoarele secţiuni:

- **Introducere** – unde se va reflecta, pe scurt, contextul şi scopul principal al studiului;
- **Materiale şi metode –** cum a fost realizat studiul şi ce teste statistice au fost aplicate;
- **Rezultate** – prezintă rezultatele principale ale studiului;
- **Concluzii** – o scurtă trecere în revistă a constatărilor făcute, cu posibile implicări pentru studii ulterioare.

Nu utilizaţi abrevieri şi citaţii în rezumatul articolului.

Cuvintele cheie

Enumeraţi 4-10 cuvinte cheie, care sunt reprezentative pentru conţinutul articolului. Pentru a uşura găsirea articolului Dvs. de către motoarele de căutare ale bazelor de date, folosiţi termeni recomandaţi din lista de titluri cu subiect medical de pe http://nlm.nih.gov/ mesh.

Înregistrarea trialului clinic

În caz dacă articolul Dvs. comunică rezultatele unui trial clinic,

ples of essential contribution to the work are: developing of the study design, patients recruitment, participation in data collection, data analysis, interpretation of results, writing of the manuscript, performing of the tests, pictures taking, drawing conclusions. The authors list should not exceed 10 persons. If the research group exceed 10 individual authors, in the "Authors name" section first two will be cited, all others should be mentioned at the end of the article, in the "Acknowledgements and funding" section.

Members of the research group who do not meet the formal criteria of the authorship, but have had some contribution to the paper, may be mentioned in the "Acknowledgements and funding" section.

Note: To differentiate the corresponding author, as well as authors who have an equal contribution to the work, using special characters as a superscript index at the end of their names is recommended:

(*) – Corresponding author;

(†) – Authors with equal contribution. (e.g. Adrian Belii*, Adrian Belii†)

• **Affiliation:** Please state the full name of institution, city and country to which the author(s) is affiliated. Affiliation should be marked with Arabic numerals in superscript after the author(s) name (e.g. Adrian Belii[1])

- **Article highlights:**
  - What is not yet known on the issue addressed in the submitted manuscript (described in 1-3 sentences)
  - The research hypothesis (described in 1-2 sentences)
  - The novelty added by manuscript to the already published scientific literature (limited to 1-3 sentences).

From new page:

Abstract

The abstract should be written using the past tense, third person. It should provide a concise summary of the purpose, objectives, significant results and conclusions of the study. The summary text should not exceed 350 words organized into the following sections:

- **Introduction** – reflect in short the context and purpose of the study;
- **Materials and methods** – describe how the study was conducted and specify the applied statistics;
- **Results** – present the key results of the study;
- **Conclusions** – a brief overview of the findings, with possible implications for further studies.

Do not use abbreviations or citations in the abstract of the article.

Key words

List 4-10 keywords that are representative for the contents of the article. To facilitate finding of your article by search engines of electronic databases, use MESH keywords list (available on http:// nlm. nih.gov/mesh).

Registered clinical trial

In case if your article reported the results of a clinical trial, please indicate Trial Register and the unique registration number of the trial.

vă rugăm să indicaţi Registrul trialului şi numărul unic de înregistrare a trialului.

Exemplu: *„Current Controlled Trials ISRCTN61362816”*. Atenţie! Nu trebuie să existe niciun spaţiu între literele şi cifrele numărului unic de înregistrare a trialului. Pentru mai multe informaţii, va rugam să accesaţi http://www.isrctn.org (*International Standard Randomised Controlled Trial Number*) şi http://www.icmje.org (*International Committee of Medical Journal Editors*).

Din pagină nouă:

Introducerea

Introducerea, scrisă la timpul trecut, persoana a treia, trebuie:

- să ofere informaţii care ar permite cititorilor din afara domeniului să intre în contextul studiului, să-i înţeleagă semnificaţia;
- să definească problema abordată şi să explice de ce aceasta este importantă;
- să includă o scurtă trecere în revistă a literaturii recente din domeniu;
- să menţioneze orice controverse sau dezacorduri relevante în domeniu;
- să formuleze ipoteza de cercetare şi să prezinte parametrul principal şi cei secundari de rezultat;
- să concludă cu scopul lucrării şi cu un comentariu care să ateste dacă scopul propus a fost atins.

Materiale şi metode

În secţiunea „Materiale şi metode” trebuie să fie descrise cu detalii suficiente procedurile efectuate. Aici se vor menţiona protocoalele detaliate privind metodele utilizate precum şi informaţii justificative. Se vor include: *design*-ul studiului, descrierea participanţilor şi materialelor implicate, descrierea clară a tuturor intervenţiilor şi comparaţiilor efectuate, precum şi testele statistice aplicate. Se vor specifica denumirile generice de medicamente. Atunci când în cercetare sunt folosite branduri, se indică în paranteze denumirea lor comercială. În cazul studiilor pe subiecţi umani sau pe animale, trebuie să fie menţionată aprobarea etică (data şi nr. procesului verbal al şedinţei Comitetului de Etică, preşedintele CE şi denumirea instituţiei, în cadrul căreia activează CE), precum şi consimţământul informat al persoanelor.

Rezultate

Rezultate şi discuţiile vor fi prezentate în secţiuni separate.

Autorii trebuie să prezinte rezultate clare şi exacte. Rezultatele prezentate trebuie explicate (nu justificate sau comparate, în această secţiune) cu constatări fundamentale, evident, referitoare la ipoteza care a stat la baza studiului. Rezultatele trebuie redate concis şi logic, cu accentuarea celor noi.

Discuţii

Se va descrie impactul, relevanţa şi semnificaţia rezultatelor obţinute în domeniul respectiv. Rezultatele obţinute se vor compara cu cele provenite din studiile anterioare din domeniu şi se vor trasa potenţiale direcţii viitoare de cercetare. Discuţiile trebuie să conţină interpretări importante ale constatărilor şi rezultatelor, în comparaţie cu studiile anterioare. De asemenea, se vor menţiona limitele studiului şi factorii potenţiali de *bias*.

Concluzii

Această secţiune trebuie să concludă laconic întregul studiu şi

E.g.: “Current Controlled Trials ISRCTN61362816”

Attention! There should be no space between letters and numbers of the unique record number of the trial. For more information, please visit http://www.isrctn.org (International Standard Randomized Controlled Trial Number) and http://www.icmje.org (International Committee of Medical Journal Editors).

From new page: Introduction

The Introduction section should be written using past tense, third person, and should:

- provide information that would allow readers outside of the field to enter the context of the study, to understand its meaning;
- define the problem addressed and explain why it is important;
- include a brief review of recent literature in the field;
- mention any controversy or disagreement existing in the field;
- formulate research hypothesis and present the main and secondary assessed outcomes;
- conclude with the research’ propose and a short comment whether the purpose has been achieved.

Materials and methods

“Materials and methods” section should present in sufficient details all carried out procedures. Here should be described protocols and supporting information on the used methods. It will include study design, subjects’ recruitment procedure, clear description of all interventions and comparisons and applied statistics. In the manuscript text the generic names of drugs should be used. When drug brands are used their trade name will be shown in parentheses. For studies on humans or animals a statement about ethical approval and informed consent of study subjects should be include. Please specify date and number of Ethics Committee (EC) decision, chair of the EC as well as institution within EC is organized.

Results

Results and discussion should be presented in separate sections. Authors must present results in a clear and accurate manner. Results should be explained (not justified or compared in this section) and include fundamental statements related to hypothesis behind the study. The results should be presented concisely and logically, emphasizing on new original data.

Discussions

Describe the impact, relevance and significance of the obtained results for the field. The results are compared with those from previous publications and draw potential future research directions. Discussions should include important interpretations of the findings and results compared with previous studies. Also, study limitations and potential bias should be mentioned.

Conclusions

This section should conclude laconically entire study, and highlight the added-value brought on the studied issue. The conclusions should not provide new information or double (repeat) those presented in the “Results” section.

să specifice, care este plus-valoarea adusă la informaţiile disponibile despre subiectul abordat. În concluzii nu se vor oferi informaţii noi şi nu se vor dubla (repeta) cele prezentate în secţiunea „Rezultate".

Abrevieri

Folosiţi numai abrevieri standard. De asemenea, pot fi formulate şi alte abrevieri, cu condiţia că acestea vor fi descifrate în text atunci când sunt utilizate pentru prima dată. Abrevierile din figuri şi tabele vor fi descifrate în legendă. Abrevierile trebuie folosite cât mai rar posibil.

Declaraţia de conflict de interese

După publicare, persoanele sau organizaţiile implicate în studiu vor deveni publice şi astfel poate fi influenţată reputaţia lor. Prin urmare, autorii trebuie să dezvăluie relaţia financiară sau non-financiară cu persoane sau organizaţii şi să declare conflictele de interese pentru datele şi informaţiile prezentate în manuscris. În conformitate cu ghidurile ICMJE, Autorul (autorii) trebuie să completeze o declaraţie privind Conflictele de interese, care va fi prezentată la sfârşitul articolului publicat.

Completând declaraţia referitoare la Conflictele de interes, se vor lua în consideraţie:

Pentru Conflicte de interese financiare

- specificaţi dacă vreo organizaţie are relaţie financiară cu lucrarea ştiinţifică reflectată în manuscris, inclusiv de finanţare, salariu, rambursări;
- menţionaţi, dacă articolul are un impact asupra organizaţiei date, ce ar genera pierderi sau profituri după publicare, în prezent sau în viitor;
- autorul (autorii) trebuie să precizeze dacă deţin cote de proprietate în orice organizaţie care ar putea să suporte pierderi sau să aibă profituri după publicare, în prezent sau în viitor. De asemenea, se recomandă să se specifice dacă autorul (autorii) deţin(e) sau aplică pentru orice drepturi de proprietate (brevet) în legătură cu conţinutul utilizat în manuscris;
- precizaţi dacă există oricare alte conflicte de interese.

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- Vă rugăm să specificaţi oricare conflicte de interese non-financiare legate de politică, individuale, religioase, ideologice, educaţionale, raţionale, comerciale etc., care au legătură cu manuscrisul.

Contribuţia autorilor

Această secţiune a manuscrisului are rolul de a specifica contribuţia şi gradul de implicare a fiecărui autor. În acest sens, vă rugăm să respectaţi formatul exemplului propus: *„HW a conceput studiul, a participat la design-ul studiului şi a ajutat la redactarea manuscrisului. MG a efectuat procesarea exemplarelor, a metodelor de cultură ale ţesutului şi a elaborat manuscrisul. TK a efectuat testele de imunofluorescenţă. PN a participat la colorarea probelor şi la analiza citometrică prin flux. AR a participat la elaborarea design-ului studiului şi a efectuat analiza statistică. Manuscrisul final a fost citit şi aprobat de către toţi autorii".*

Fiecare Autor trebuie să aibă o contribuţie individuală în desfăşurarea cercetării, pregătirii manuscrisului şi publicării lucrării. Un Autor trebuie să contribuie semnificativ la conceptul şi *design*-ul lucrării, la efectuarea procedurilor experimentale, la colectarea datelor, la compilarea, analiza, interpretarea şi validarea rezultatelor.

Conform recomandărilor Comitetului Internaţional al Editorilor Revistelor Medicale, ICMJE, (www.icmje.org), drept autor poate fi considerată persoana care se încadrează în toate cele 4 criterii:

Abbreviations

Use only standard abbreviations. Other abbreviations may be defined and provided when are used for the first time in the manuscript. Abbreviations in the figures and tables will be explained in legend. Abbreviations should be used as rare as possible.

Declaration of conflict of interests

Following publication, persons or organizations involved in the study become public and thus their reputation may be influenced. Therefore, authors must disclose financial and non-financial relationship with people or organizations and to declare conflicts of interest related to the data presented in the manuscript. In accordance with the ICMJE guidelines, authors must fulfill a statement of conflicts of interest, which will be published at the end of the article.

Complementing the declaration of conflicts of interest the following will be taken into consideration

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Authors' contributions

This section of the manuscript is to specify the input and involvement of each author. In this regard, please follow the suggested format: *"HW conceived the study and participated in study design and helped drafting the manuscript. MG performed the processing of specimens and tissue culture methods and drafted the manuscript. TK performed immunofluorescence tests. PN participated in staining and flow-cytometry. AR participated in the study design and performed the statistical analysis. Final manuscript was read and approved by all authors".*

Each author must have an individual contribution to the research, manuscript preparation and work publication. An author should contribute substantially to one of the following: the concept and design of the work, performing of the experimental procedures, data collection, compilation, analysis, interpretation and validation of results.

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o has made a substantial personal contribution in desi-gning,

- o a adus o contribuţie individuală substanţială conceperii, elaborării design-ului cercetării, sau a colectat, analizat sau interpretat datele;
- o a elaborat manuscrisul sau l-a revăzut în mod critic, aducând o contribuţie intelectuală importantă;
- o a aprobat versiunea finală a manuscrisului, gata pentru publicare;
- o este de acord să fie responsabilă pentru toate aspectele legate de cercetarea efectuată şi de manuscrisul depus pentru publicare şi să dea asigurare, că toate întrebările referitoare la acurateţea sau integritatea lucrării vor investigate şi rezolvate în mod corespunzător.

Notă: Persoanele, care au contribuit la realizarea lucrării, însă nu se încadrează în toate cele 4 criterii enunţate mai sus, nu pot fi considerate drept autori; contribuţia acestora va fi menţionată în secţiunea „mulţumiri şi finanţare" a manuscrisului. De asemenea, persoanele care au fost implicate doar în colectarea datelor, supraveghere, asistenţă tehnică şi finanţare, nu deţin drept de Autor, dar ei pot fi menţionaţi în secţiunea „mulţumiri şi finanţare". Simpla deţinere a funcţiei de şef de unitate, departament sau instituţie, în cadrul căreia s-a efectuat cercetarea, fără îndeplinirea tuturor celor 4 recomandări ale ICMJE, nu oferă dreptul de a fi (co)autor al lucrării.

Mulţumiri şi finanţare

Persoanele care au contribuit la elaborarea design-ul studiului, colectarea datelor, analiza şi interpretarea acestora, la pregătirea manuscrisului şi la redactarea lui critică, au oferit suport general sau tehnic, au contribuit cu materiale esenţiale pentru studiu, dar care nu îndeplinesc criteriile ICMJE de Autor, nu vor fi considerate drept Autori, dar contribuţia lor va fi menţionată în secţiunea „mulţumiri şi finanţare". Tot în această secţiune se vor menţiona sursele de finanţare ale lucrării. Menţionarea persoanelor fizice sau juridice, care au contribuit la realizarea lucrării şi manuscrisului, poate fi făcută doar după obţinerea unei permisiuni de la fiecare dintre ele.

Tabelele

Fiecare tabel va fi creat cu dublu-spaţiere şi amplasat pe o pagină separată, după textul manuscrisului. Enumerarea tabelelor va fi consecutivă, cu cifre arabe, în ordinea primei lor citări în text, scris cu caractere grase **(bold)**, alinierea – pe stânga, deasupra tabelului. Fiecare tabel va avea un titlu laconic, care va fi scris cu caractere normale (regular) sub numărul tabelului. Nu utilizaţi caractere bold în interiorul tabelului. Urmaţi exemplul prezentat:

Tabelul 1
Evenimente adverse intra-anestezice şi imediat post-extubare

	Lot experimental (n=100)	Lot control (n=100)	p
Disritmii	6,0%	3,0%	0,49
Instabilitate hemodinamică	7,0%	1,0%	0,034
Trezire prelungită*	11,0%	4,0%	0,19
GVPO† post-extubare	8,0%	27,0%	0,007
Durere intensă la trezire	17,0%	19,0%	1,0

Notă: * – trezire neobişnuit de lentă, după ce concentraţia cerebrală a reziduurilor de anestezice a trecut sub pragul de inducere a hipnozei; † – greaţă şi vomă postoperatorie. Analiza statistică utilizată: testul Fisher.

- o developed or reviewed critically the manuscript bringing a significant intellectual contribution;
- o approved the final version of the manuscript ready for publication;
- o agrees to be responsible for all aspects of the conducted research and submitted manuscript and to assure that all questions relating to accuracy or completeness of the work was adequately assessed and resolved.

Note: Persons who have contributed to the work, but not fit the four criteria mentioned above cannot be considered as authors. Their contribution will be mentioned in the "Acknowledgment and funding section" of the manuscript. Also, people who have only been involved in data collection, monitoring, technical assistance and funding, are not eligible as coauthors, but they may be mentioned in the "Acknowledgements and funding" section. Mere position of head of unit, department or institution, on which the research was conducted, without fulfilling all four ICMJE criteria, doesn't provide the right to be a coauthor of the work.

Acknowledgements and funding

People who contributed to the study design, data collection, analysis and interpretation, manuscript preparation and editing, offered general or technical support, contributed with essential materials to the study, but do not meet ICMJE authorship criteria will not be considered as authors, but their contribution will be mentioned in section "Acknowledgements and funding". Also in this section must be specified the sources of work funding. Mention of persons or institutions who have contributed to the work and manuscript can be made only after obtaining permission from each of them.

Tables

Content of each table should be double-spaced and placed on a separate page after the text of the manuscript. Tables numbering will be done using consecutive Arabic numerals in the order of their first citation in the text; it is should be written in **bold**, align to left and place above the table. Each table should have a concise title that will be written in bold (regular) under table number. Do not use bold within the table. Please follow the example:

Tabelul 1
Intra-anesthetic and immediately post-extubation adverse events

	Experimental Cohort (n=100)	Control Cohort (n=100)	p
Dysrhythmia	6,0%	3,0%	0,49
Hemodynamic instability	7,0%	1,0%	0,034
Prolonged awakening*	11,0%	4,0%	0,19
PONV† post-intubation	8,0%	27,0%	0,007
Strong pain on awakening	17,0%	19,0%	1,0

Note: * – Unusually slow awaking, after that cerebral concentration of the anesthetic reach the under hypnotic level; † – postoperative nausea and vomiting. Used statistical analysis: Fisher's exact test.

Legendele şi notele explicative vor fi făcute sub tabel. Toate abrevierile non-standard se vor explica în notele de subsol, folosind următoarele simboluri, în următoarea ordine: * , † , ‡ , § , | | , , ** , † † , ‡ ‡ , § § , | | | | , ¶ ¶ etc.

Menţionaţi, de asemenea, testele statistice aplicate şi tipul de date prezentate. Asiguraţi-vă că fiecare tabel este citat în text. Dacă utilizaţi date din altă sursă publicată sau nepublicată, trebuie să obţineţi permisiunea şi să declaraţi pe deplin sursa sub tabel.

Figurile

Figurile vor fi prezentate atât în manuscris, cât şi pe fişiere separate. În manuscris, figurile vor fi prezentate după textul lucrării, fiecare pe pagină separată şi vor fi numerotate consecutiv, cu cifre arabe, în ordinea citării lor în text. Numerotarea va fi scrisă abreviat (**Fig. 1**), cu caractere grase (**bold**), alinierea – pe stânga, sub figură. Fiecare figură va avea un titlu laconic, care va fi scris cu caractere normale (regular) în dreptul numerotării.

Figurile trebuie să fie calitative, vizibile în detaliu. Fotografiile cu persoane potenţial identificabile trebuie să fie însoţite de permisiunea scrisă de a utiliza fotografia. În caz contrar, faţa persoanelor trebuie acoperită cu o bandă neagră. În cazul în care o figură a fost publicată anterior, faceţi referinţă la sursa originală şi prezentaţi permisiunea scrisă de la deţinătorul drepturilor de autor pentru a reproduce figura. Permisiunea poate fi luată atât de la autorul figurii, cât şi de la editor, cu excepţia documentelor din domeniul public.

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- o EPS (format preferat pentru diagrame)
- o PowerPoint (figurile trebuie să fie de mărimea unui singur diapozitiv)

Titlul fişierului va consta din numărul figurii şi un titlu scurt, identificabil.

Legendele figurilor

Legenda figurii va fi scrisă în continuare, imediat după titlul figurii. Descrierea figurii nu trebuie să repete descrierea din textul manuscrisului. Când sunt folosite simboluri, săgeţi, numere sau litere pentru a identifica, descrie părţi ale ilustraţiilor, identificaţi-le şi explicaţi-le pe fiecare în mod clar în legendă. Explicaţi scala internă şi identificaţi metoda de colorare în microfotografii.

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Toate referinţele bibliografice trebuie să fie numerotate consecutiv, între paranteze pătrate [ ], în ordinea în care sunt citate în text. Citatele de referinţă nu trebuie să apară în titluri sau subtitluri. Fiecare referinţă trebuie să aibă un număr individual. Citările multiple din cadrul unui singur set de paranteze trebuie să fie separate prin virgulă şi spaţiu. În cazul în care există trei sau mai multe citări secvenţiale, acestea ar trebui să fie indicate sub formă de serie. Exemplu: [1, 5-7, 28].

Vă rugăm să evitaţi folosirea excesivă a referinţelor. În cazul în care se folosesc sisteme automate de numerotare, numerele de

Legends and notes will be place under the table. All non-standard abbreviations should be explained in footnotes, using the following symbols, in the following order: *, †, ‡, §, | |, ¶, **, † †, ‡ ‡, § §, | | | |, ¶ ¶ etc.

Applied statistical tests and the type of presented data should be also mentioned. Make sure that each table is cited in the text. If you use data from another published or unpublished source, you must obtain permission and cited the source below the table.

Figures

Figures will be included in the main manuscript, and also submitted as separate files. The manuscript figures should be presented, each one on a separate page and should be numbered consecutively with Arabic numerals in the order of their citation in the text. Figure numbering will be written abbreviated (Fig. 1), using bold fonts, left alignment, and placed under the figure. Each figure should have a laconic title that will be written using regular font and place in the right of the figure's number. Figures' quality should assure the visibility of details. Pictures of persons potentially identified must be accompanied by written permission to use it. If a figure has been previously published, please cite the original source and submit the written permission to reproduce the figure from the copyright owner. Permission can be taken from both the author and the publisher, except the documents of public domain.

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The file title should include the figure number and an identifiable short title.

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Figure's legend should be written immediately after the figure's title. Figure's description should not repeat the description in the text of the manuscript. When used symbols, arrows, numbers or letters to describe parts of the figure, explain clearly each one of them in the legend. Explain the internal scale and identify the staining method of the photomicrographs.

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PENTRU NOTIȚE

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