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Nicolae Testemitsanu State University of Medicine and Pharmacy

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al Republicii Moldova.
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*Energie de alternativă
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metabolismul energetic**



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**Previne complicațiile
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GEDEON RICHTER

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Issued bimonthly

Vol. 59, No 6
December 2016**Welcome to the scientific and medical journal
Curierul Medical!**

From its debut in 1958 the journal has striven to support the interests of Moldovan medicine concerning the new concepts of its development. The Editorial Board warmly welcomes both the readers of and the authors for the journal, all those who are enthusiastic in searching the new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution into the science of medicine will find our journal helpful and encouraging.

The journal is accredited by the National Council for Accreditation and Attestation. The journal publishes official papers, scientific articles, editorials, clinical studies and cases, lectures, methodological guides, reviews, brief reports and correspondence. The journal welcomes articles in English, Romanian and Russian. The journal editorial policy provides the prompt publication of papers within 12 weeks after receiving them.

**Bine ați venit la revista științifică medicală
Curierul Medical!**

De la prima apariție în 1958, revista susține și dezvoltă noile idei în domeniul medicinei, în Republica Moldova. Colegiul de redacție agreează cu multă considerație atât cititorii cât și autorii articolelor, pe toți acei care cu mult entuziasm caută noi și mult mai efective metode de soluționare a multelor probleme ale medicinei. Sperăm, că toți acei care doresc să-și aducă aportul la dezvoltarea științelor medicale, vor găsi revista noastră utilă și atractivă.

Revista este acreditată de către Consiliul Național de Acreditate și Atestare. Revista publică comunicări oficiale și, totodată, sunt editate diverse publicații, inclusiv independente: articole științifice, editoriale, cercetări și prezentări de cazuri clinice, prelegeri, îndrumări metodice, articole de sinteză, relații scurte, corespondențe și recenzii. Revista publică articole în limba engleză, română și rusă. Politică de editare a revistei prevede examinarea operativă și publicarea articolelor timp de 12 săptămâni după înaintare.

**Добро пожаловать в научно-медицинский журнал
Curierul Medical!**

С первого дня своего выпуска в 1958 году журнал стремится поддерживать и развивать новые идеи в области медицины в Молдове. Редакционная коллегия всегда рада как читателям, так и авторам статей, всем тем, кто с энтузиазмом ищет новые, более эффективные способы решения многочисленных задач медицины. Мы надеемся, что все те, кто хотят внести свой вклад в медицинскую науку, найдут наш журнал полезным и вдохновляющим.

Журнал аккредитован Высшей Аттестационной Комиссией Республики Молдова. В журнале печатаются официальные материалы, научные статьи, наблюдения из клинической практики, обобщающие статьи, краткие сообщения, методические указания, рецензии и корреспонденция. В журнале публикуются статьи на английском, румынском и русском языках. Издательская политика журнала предусматривает оперативное рассмотрение и публикацию статей в среднем в течение 12 недель после поступления.

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RESEARCH STUDIES

Legal aspects in detecting counterfeit medicines

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Abstract

Background: With the rapid development of the pharmaceutical industry cases of counterfeit medicines are more and more frequent. This is a real crime that influences Public Health, counterfeit medicines being harmful and even fatal.

Material and methods: For this study we carried out an objective analysis of the world legislative framework, with predilection for the European and national one. As landmarks served: setting the research objectives, data collection, data analysis, formulation of results and conclusions.

Results: The number of counterfeiting incidents during 2011 – 2015 constituted: 2011 – 1986 crimes, 2012 – 2018 crimes, 2013 – 2193 crimes, 2014 – 2177 crimes, 2015 – 3002 crimes. So far numerous directives have been approved, laws have been adopted and measures have been taken to stop the counterfeiting of medicines (Directive 2011/62/EU of 8 September 2011, the Convention MEDICRIME, Resolution 65.19 of the World Health Assembly, Rapid Alert System, global and regional operations of Interpol).

Conclusions: Counterfeiting of pharmaceuticals is a global problem that involves: institutions, law enforcement, customs, doctors, pharmacists, patients and citizens. The competent national authorities in collaboration with the associations of doctors, pharmacists and patients must lead risk awareness campaigns regarding purchasing of pharmaceutical products from illegal sources. Patients should be informed of the risks they assume by purchasing drugs from different websites. The population should know that websites that sell drugs must have links directing to the competent national authority or the website of European Medicines Agency or Medicines and Medical Devices Agency from the Republic of Moldova.

Key words: counterfeit medicines, directives, operations, cooperation.

Introduction

With the continuous development of the pharmaceutical industry cases of counterfeit medicines are more and more frequent.

Although the counterfeit medicines manufacture was focused in first place on drugs that don't treat diseases but only improve the quality of life, the manufacturing industry has expanded to cover any type of drugs [1, 2].

Counterfeiting medicines is a real crime, and its severity is not determined only by economic losses which is the main problem for counterfeits in other industries. The drug counterfeit is not only an economic issue but also a Public Health one [3].

Counterfeit medicines are ranked from useless to potentially dangerous. They often contain wrong level of active ingredient – too little, too much or not at all – or an active ingredient intended for a different purpose. In some cases counterfeit drugs containing highly toxic substances such as rat poison were found. In all these scenarios, the person using counterfeit drug puts his health, even his life in danger [4]. Medical products counterfeiting and similar crimes should be classified as criminal actions because of the risk they pose to public health. Disease treatment is delayed due to ineffective counterfeit drugs and illegal products, so the appropriate treatment could be futile because it got too late. Counterfeit medical products and similar crimes are silent killers because the patient could die from

illness ineffectively treated. In such cases, no one will look for counterfeiting as a possible cause [2].

WHO estimates that up to 1 percent of medicines available in developed countries are likely to be counterfeited. This figure rises to 10 percent globally, but in some areas of Asia, Africa and Latin America counterfeit medicines are up to 30 percent of the market.

Counterfeiting not only applies to drugs intended for lifestyle, including erectile dysfunction and weight loss, but also vital drugs are more and more often falsified, including those for cancer treatment, cardiac disorders and other severe illness cure [4].

The international and national legal framework shall play the central role in the combat of this inhuman act. So far numerous directives have been approved, laws have been adopted and measures have been taken to stop the counterfeiting of medicines. Are they sufficient?

Global cooperation is the key to efficient fight with counterfeit medicines. A positive aspect in this regard is the involvement and contribution of the Republic of Moldova in this fierce battle against the epidemic of counterfeit medicines.

Material and methods

For this study we carried out an objective analysis of the world legislative framework, with predilection for the European and national one. As landmarks served: setting

the research objectives, data collection, data analysis, formulation of results and conclusions. Data from official sources were studied, such as Quality Control of Medicines Laboratory (QCML), World Health Organization (WHO), Pharmaceutical Security Institute (PSI), European Directorate for the Quality of Medicines (EDQM), INTERPOL, etc. The following methods were used for the analysis: comparative and graphical representation.

Results and discussions

Pharmaceutical legislation was adopted, for the first time 40 years ago and principles of marketing, manufacturing, import, export and distribution as they are today – were adopted in 1975. WHO began fighting counterfeit medicines since the 80s, when manufacturing, distribution and drugs marketing lines were developing. The legislative framework so far has undergone various changes, for better resistance against counterfeiting.

It is very important to make the distinction between substandard and counterfeit drugs. According to WHO a counterfeit medicine is one which is deliberately and fraudulently mislabelled with respect to identity and/or source. Counterfeiting can apply to both branded and generic products and counterfeit products may include products with the correct ingredients or with the wrong ingredients, without active ingredients, with insufficient (inadequate quantities of ingredient(s) or with fake packaging. Substandard medicines (also called out of specification (OOS) products), according to the same source, are genuine medicines produced by manufacturers authorized by the National Medicines Regulatory Authority (NMRA) which do not meet quality specifications set for them by National standards [1].

In this connection we can talk about several types of counterfeiting:

- The drug is perfect imitation of the authentic version.

The same active substance and excipients, the correct amounts of API and properly packaged.

- The drug is identical to the authentic version but the amount of active substance differs from that stated on the label and the information related to the source of raw material, validity date, bioavailability doesn't correspond.
- The drug is similar to the authentic version but contains neither active nor harmful substance.
- The medicine appears at first glance similar to the authentic version but does not contain the same active substance and contains harmful substances.
- The drug packaging is fake.

Even if they are of appropriate quality and contain the correct amount of active ingredient, counterfeit medicines do not guarantee quality, as they are not manufactured in accordance with good manufacturing practice (GMP) and good distribution practice (GDP) established globally and imposed by the pharmaceutical industry to products. Counterfeit products do not equalate their safety, quality and efficacy, characteristic to genuine products [3, 5].

In recent years, according to statistics, we see a continuous growing of crimes in the field of counterfeit drugs, in the chart below are presented the details regarding the number of crimes in pharmaceutical field during the last five years, according to PSI (fig. 1).

To understand better the magnitude of counterfeiting incidents during 2015, PSI continues to monitor the quantity of seized medicines at every action of law enforcement. Any incident, which involves the seizure of more than 1000 dosage units, is classified as commercial incident. The incident involving less than 1000 dosage units is classified as non-commercial one (fig. 2).

All continents and countries are involved in medicines counterfeiting, either directly through the manufacturing, marketing, distribution of counterfeit medicines, or indi-

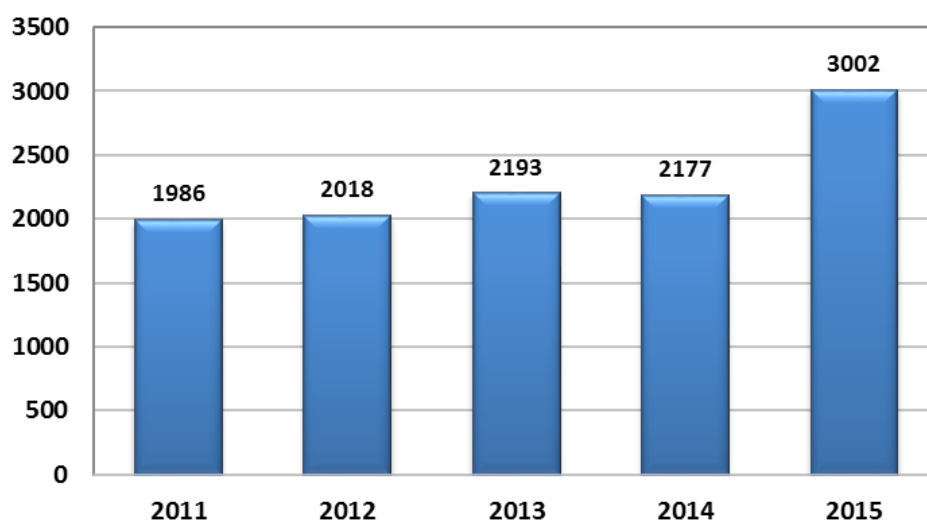


Fig. 1. The total number of counterfeiting incidents during the period 2011-2015 [6].

Counterfeit Seizures CY 2015

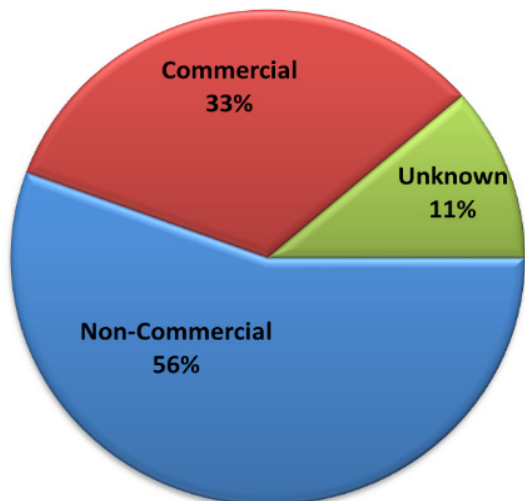


Fig. 2. Confiscation of Counterfeit Medicines, 2015 [6].

rectly through their transit through the territory of that country. If we analyze the crime incidence by continents (fig. 3), we see that the leader of counterfeit medicines is Asia – 1100 crimes, North America is ranked 2nd in the range with 779 crimes, followed by South America – 494 crimes and Europe with 358 crimes. Data were collected and analyzed by PSI.

If we consider the incidence of counterfeiting by therapeutic groups we see that the 3002 crimes that occurred in 2015 involved 1095 different pharmaceutical products. The number of products found in only one incident ranged from one drug to thirty-seven different drugs. The data showed that medicines for urogenital system, anti-infectives and for central nervous system are main therapeutic categories with the highest number of counterfeiting incidents. These three categories were determined as drugs

most frequently subject to the pharmaceutical counterfeiting. While the ranks of the top therapeutic categories were relatively unchanged, the Institute has noted seven therapeutic categories that have had a percentage increase on a year-to-year basis. Specifically, the genito-urinary therapeutic category led with the largest percentage increase at sixty-five percent (+65%). Categories with percentage increases also included dermatologicals (+57%), cytostatics (+29%), cardiovascular (+29%), respiratory (+28%), CNS (+11%), and alimentary (+4%) (fig. 4) [6].

More and more people are buying drugs and medical devices over the Internet through online pharmacies and auction sites. Unfortunately, a large number of these Internet sites are not licensed and regulated and sell fake or substandard products.

If an online provider hides physical address, it is a warning sign that their products could be dangerous. WHO estimates that 50 percent of medicines available from such websites are counterfeit.

In particular, the purchase of drugs only on prescription from unlicensed or suspicious sources significantly increases the risk of getting substandard or fake products. It is important to consult a medical professional in order to obtain medicines from a regulated source.

Buying medicines online may seem cheaper, faster and more convenient, but the dangers are greater than the benefits [4].

In many countries it is legal to purchase drugs and medical products from authorized online pharmacies with a prescription if necessary. However, tens of thousands of websites provide drugs illegally. Combined with rising consumer demand, technological progress, namely the widespread use of the Internet, advertising, the sale and supply of illicit and counterfeit drugs through unauthorized and unregulated websites became a global and continually expanding problem.

Incidents - Regions of the World

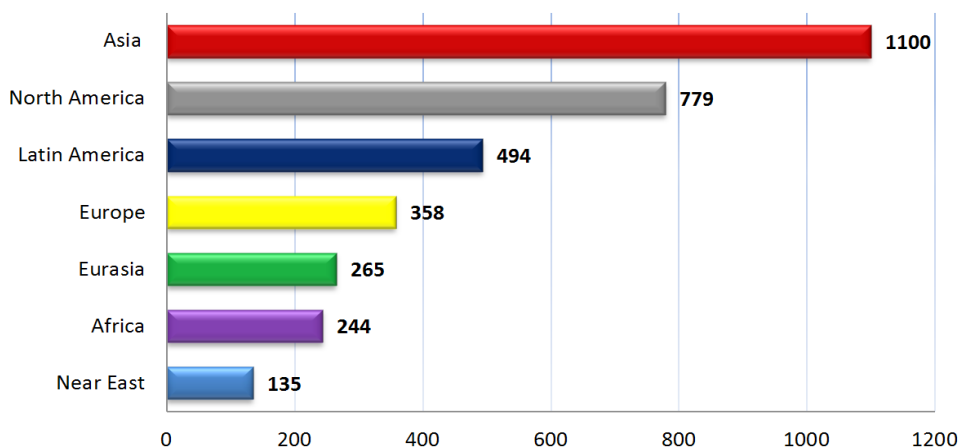


Fig. 3. The incidence of counterfeiting by continents, 2015 [6].

The report “A study of illicit online marketplaces” of Interpol analyzes the extent of the pharmaceutical delinquency problem on Darknet, through a case study of some of the largest online markets of counterfeit medicines: Silk Road 2.0 (which was later suspended by authorities of law enforcement) and Evolution Marketplace (which still works).

More than 10000 advertisements are posted in the section entitled “Medicines” on Silk Road 2.0. When we divide these numbers we find that the highest proportion of products published on Silk Road 2.0 refers to prescription medications. Rx drugs constitute 30 percent of the total amount of medicines advertisements on Silk Road 2.0, cannabis being the second product after its share – 17 percent (fig. 5) [7].

In order to lead a successful fight with continuously growing crimes, in 2011 was approved Directive 2011/62/EU of the European Parliament and of the Council of 8 June 2011 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use, as regards the prevention of the entry into the legal supply chain of falsified medicinal products. This Directive contains rules concerning the extension of rules regarding sales agents, strengthening of intermediaries obligations, improvement of quality control of API and certain excipients, regulation of imported drugs intended for re-export and establishment of new rules on access to medicines stored in warehouses and free trade zones, demand of characteristics related to the safety of medicines prone for counterfeiting, strengthening of inspections and ensuring

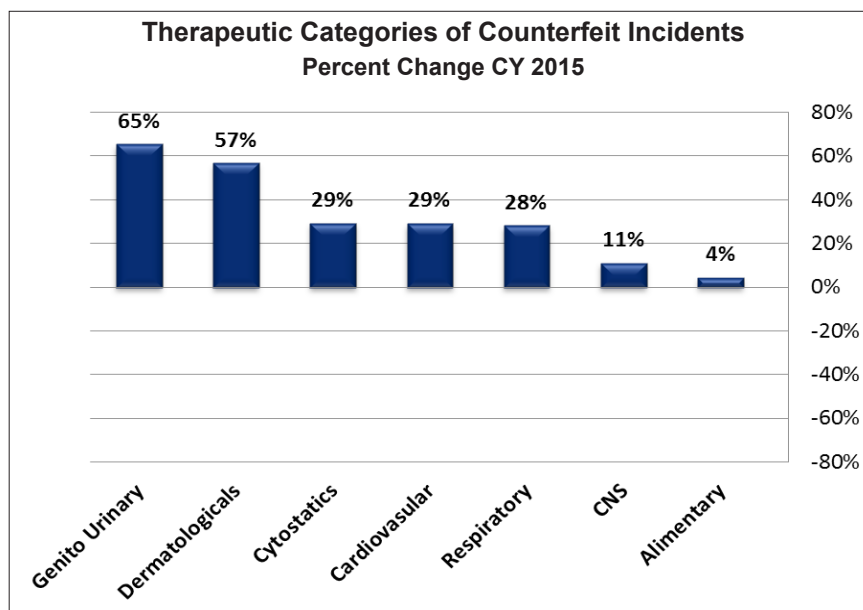


Fig. 4. Therapeutic categories of counterfeit incidents, percent change, 2015 [6].

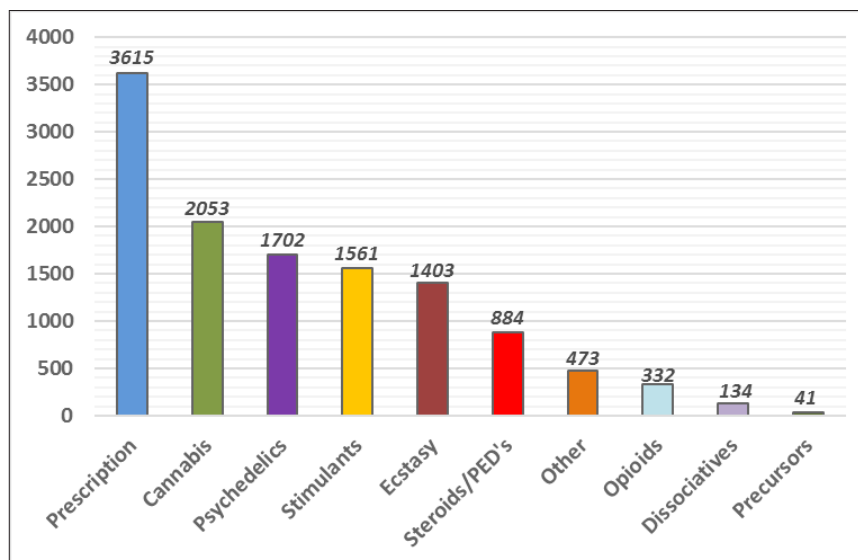


Fig. 5. Number of published products on Silk Road 2.0, by categories [7].

appropriate punishment for committing an infringement in the Member States [8].

The Council of Europe has been concerned by the lack of international law harmonization, deterrent penalties that were not proportionate to the harm caused to patients and the involvement of criminal organizations operating across borders. To this effect was approved "Council of Europe Convention on counterfeiting of medical products and similar crimes involving threats to public health", adopted in Moscow on 28 October 2011 (MEDICRIME Convention). MEDICRIME Convention is the first international criminal law instrument that obliges the states parties to criminalize:

- Manufacture of counterfeit medical products;
- Supplying, offering to supply and trafficking counterfeit medical products;
- Forging documents;
- Unauthorized manufacture or supply of medical products and the marketing of medical devices that do not conform to requirements.

The Convention provides a framework for national and international cooperation through various administrative sectors. It provides measures for coordination at national level, preventive measures aimed at public and private sectors, and measures to protect victims and witnesses. It also establishes a monitoring body responsible for tracking the implementation of the Convention by the States parties [9].

Open for seminars in 2011, the Convention has been so far ratified by 9 countries (Spain, Guinea, Hungary, Moldova and Ukraine) and signed by 26 other countries. The Convention was signed by Moldova in Vienna on September 20, 2012 and ratified on 14.08.2014 (Law no.67 of 16/04/2014 to ratify the Council of Europe Convention on counterfeiting of medical products and similar crimes involving threats to public health). MEDICRIME Convention came into force on 1 January 2016 [10, 11, 12].

The 65th Assembly of the World Health Organization, held in 2012 in Geneva, adopted resolution 65.19 that establishes the mechanism of fight against SSFFC Medical

Products (substandard / spurious / falsely-labelled / falsified / counterfeit medical products) [13]. This resolution renewed and restored a mandate to WHO and Member States in addressing SSFFC medical products in a transparent and comprehensive way from public health perspective, and expressly excluded intellectual property rights. Its objective is to work with Member States to improve the quantity, quality and analysis of accurate data on SSFFC medical products and to use this data to better prevent, detect and respond to these products in order to protect public health [15].

Member States have adopted a work plan of SSFFC, containing 8 points, focused on cooperation, collaboration and consolidation of power.

The work plan of Member States SSFFC:

1. Strengthen the capacity of regulators and quality control laboratories.
2. Enhancing collaboration between regulatory authorities;
3. Communication, education and awareness;
4. Transparent collaboration of stakeholders;
5. Identify actions, activities and behaviors resulting from SSFFC medical products.
6. Increase the ability to strengthen national and regional integrity of the supply chain;
7. Cooperation regarding the supervision and monitoring of the SSFFC medical products.
8. Collaboration within WHO regarding the access to qualitative, safe, effective and affordable health care products [13].

Global Surveillance and Monitoring System for SSFFC medical products was launched in West Africa, in July 2013. Since then, over 300 regulators from 113 member (fig 6) states have been trained in its use and 1040 SSFFC medical products have been reported [16].

Rapid Alert System for counterfeit medicines (*Rapid Alert System (RAS)*) is a web communications network involving focal persons and representatives of countries and areas from the Western Pacific region, WHO and partner



Fig. 6. Countries implimenting Rapid Alert System [16].

agencies. The aim is to warn countries, member areas and partner organizations, through focal points and representative network on cases of counterfeit medicines. Cases of counterfeit drugs can be reported through the system, using an electronic reporting form. Alternatively, reports can be submitted by any other means (e-mail or fax) to the secretariat, which will introduce immediately into the system [14]. Some Member States report suspected SSFFC medical products and other validated SSFFC medical products. Depending on the nature of the report, a moderator will disseminate information to all members of RAS. Confirmed cases are included in the database of WHO. When a report is received from WHO, it is loaded automatically into a secure database of WHO and immediately compared to all reports. WHO will contact the focal point of reporting within 72 hours for further details and if requested will provide technical support. In emergency situations this can take the form of facilitating emergency laboratory analyzes or in extreme and complex cases to send experts.

In all cases, analysts will work in focal point to gather more information about the possible SSFFC medical product in order to validate the information.

The analyst will close a case by classifying the SSFFC medical product in the following categories:

- Substandard
- Faked
- Genuine
- Not registered
- Stolen / diverted

This classification shall be carried out only if there is sufficient information to make a determination beyond doubt. During 2016 all focal points will be able to search the database through a secure link to verify if a suspected SSFFC medical product has already been reported to the WHO (fig. 7) [16].

All these are the main lines of fight against counterfeiting at national and international levels.

Interpol (International Criminal Police Organization) has a very large share in the fight against pharmaceutical crimes. This is an international cooperation organization of police forces. Created in 1923, the organization has 190 member states. It participates in combating counterfeiting of medicinal products through global and regional operations.

Operation Pangea is generally referred to as a global operation organized by Interpol in 2008 with support from the World Customs Organization and the Forum on International Crime in the pharmaceutical field. The theme of the annual operation is to combat online pharmaceutical crime and sale of medical devices. The Republic of Moldova, in collaboration with Medicines Agency and national law enforcement, participated for the first time in operation Pangea VIII in 2015. The conference in Brussels from 23-24 February was aimed at bringing together the contact points of 60 countries of the world participating in the following Pangea IX and 145 representatives from various national and global structures in this field.

Operation Pangea involves a week to prevent and combat the illicit sale of pharmaceutical products as well as medical devices online or in the market with the aim to guarantee the security of overall health and the health of patients, particularly vulnerable people: the poor, elderly, children and those sick. The problem of counterfeit and unauthorized drugs as well as medical devices is of worldwide importance. During this operation the customs, regulatory authorities of medicines and medical devices, national police and private sector companies (manufacturers and wholesale distributors) are working together. Its activity is directed towards three main components, which are used by illegal websites to develop commercial activ-

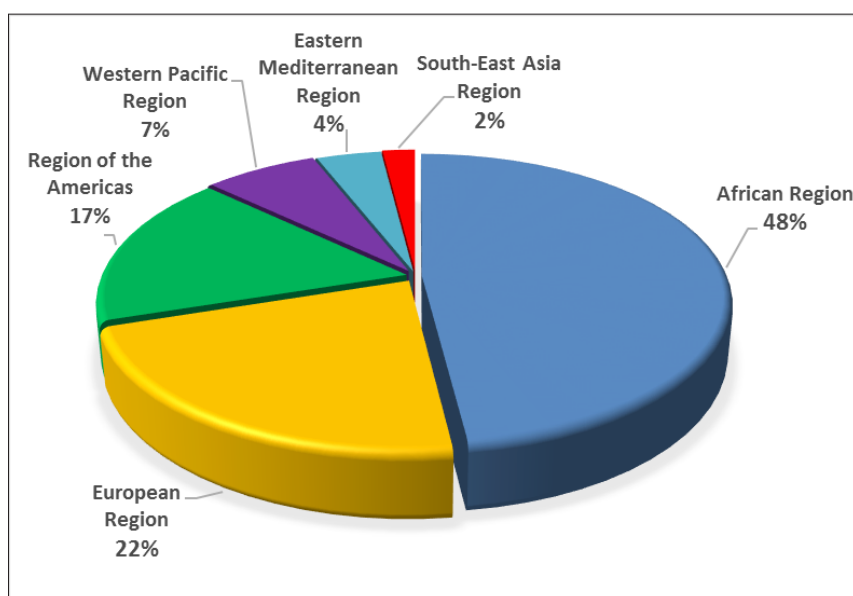


Fig. 7. Levels of current regional reporting, updated in March 2016 [16].

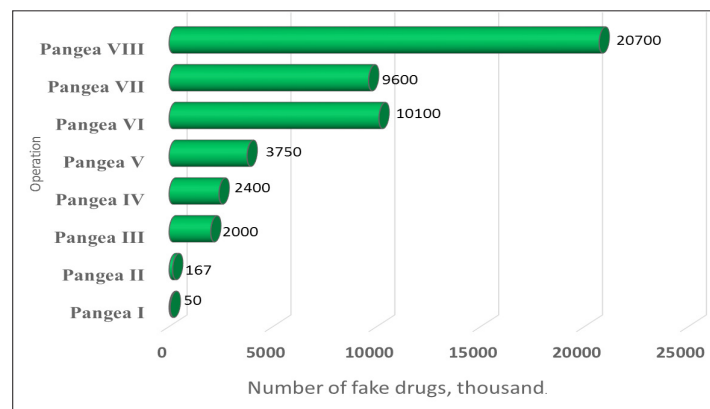


Fig. 8. The number of counterfeit medicines seized by INTERPOL [17].

ity – Internet Service Provider (ISP), payment systems and service delivery.

During the period 2008-2015 there were eight Pangea operations, 10 countries attended the first year and gradually their number grew in such a way that in 2015 there were 115 countries and representatives participating. The operation efficiency increases with the number of participants. During Pangea I operation several thousands of illegal medicines were seized, the number increased during operation Pangea II, so that in 2015 the number of seized drugs reached 20.7 mln (fig. 8).

Regional operations aim at disrupting the activities of transnational criminals involved in trafficking of illicit pharmaceuticals in the regions particularly affected by this problem, such as South-Eastern Asia (Operation-Storm) and all regions of Africa (e.g. operations Gibo, Mpili and Porcupine).

During the period 2010 – 2014, these operations led to the arrest of 1400 suspects, suspension of 57000 online illicit pharmacies and seizure of 30.3 million units of illicit drugs [17, 18].

Conclusions

1. The counterfeiting of pharmaceuticals is a global problem that involves several actors: institutions, law enforcement, customs, doctors, pharmacists, patients and citizens.

2. The competent national authorities in collaboration with the associations of doctors, pharmacists and patients must lead risk awareness campaigns regarding purchasing of pharmaceutical products from illegal sources.

3. Patients should be informed of the risks they assume by purchasing drugs from different websites.

4. The population should know that websites that sell drugs must have links directing to the competent national authority or the website of European Medicines Agency or Medicines and Medical Devices Agency from the Republic of Moldova.

5. Monitoring sites (and social networks) is vital in order to know the perception of patients about a brand or product. This monitoring is a source of information on trends in consumption and can highlight sites with counterfeits and illicit trade in real time.

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Antifungal activity of extracts from *Arthrospira platensis* against some pathogens, causing invasive mycoses

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Abstract

Background: The aim of this study was to investigate the effect of extracts from the biotechnologically obtained *Arthrospira platensis* (spirulina) biomass with metals, on some strains of filamentous fungi causing invasive mycoses.

Material and methods: The extracts from spirulina biomass were used as material. The agar diffusion method was used to identify antifungal activity of extracts. Five strains of filamentous fungi were used as test objects. The level of toxicity was evaluated based on the quantitative determination of the activity of extracellular lactate dehydrogenase (LDH).

Results: The ethanol extracts from biomass of spirulina containing chromium, copper, cadmium, cobalt, zinc and iron, have antifungal activity against *Aspergillus fumigatus* CNM-FA-02, *Mucor vulgaris* CNMN-FD-07, *Penicillium expansum* CNMN-FD-05, *Fusarium solani* and *Fusarium oxysporum*. At the same time, the extracts from standard biomass are inactive (exception: the ethanol extract slightly suppresses the growth of *Aspergillus fumigatus* CNM-FA-02). Naftifine hydrochloride was used as a control. We found four variants with higher antifungal activity than naftifine hydrochloride. In the case of biomass extracts containing copper, cobalt, chromium and cadmium the inhibition of fungal growth was associated with increased activity of extracellular LDH.

Conclusions: Extracts from biomass containing metals are characterized by various antifungal activities, inhibiting the fungal growth and increasing the release of lactate dehydrogenase into the extracellular medium.

Key words: antifungal activity, ethanol and water extracts, *Arthrospira platensis*.

Introduction

Over the last few decades, the number of patients susceptible to invasive infections caused by filamentous fungi, usually found in different natural habitats such as soil and various organic substrates, grew steadily. The list of such patients includes persons with hematologic and autoimmune diseases, subjected to organ transplant or with a compromised immune status [1]. Even in case of proper treatment, the most invasive fungal infections are associated with high rates of mortality of over 50% [2,3]. The most known agents of invasive mycoses belong to the genera *Aspergillus* and *Mucor*. In recent years, this list has been supplemented by the less common filamentous fungi, such as *Fusarium spp.* and *Penicillium spp.* There have been also expanded the limits of applicability of "fungal invasion" term from "invasive disease" to previously less recognized entities, such as severe asthma with fungal sensitization, chronic cough associated with fungal infections, allergic bronchopulmonary mycosis and allergic fungal rhinosinusitis [2,4,5].

Normally, the appropriate antifungal therapy is prescribed depending on the patient's immune status, the site of infection, the biological characteristics of the pathogen and the pharmacokinetic characteristics of the applied drug. There is utilized a limited number of antifungal medications available in the treatment of systemic fungal infections, forming four different classes [6]: (a) polyene macrolides that change the membrane functions of the pathogen; (b) azole derivatives that inhibit lanosterol 14

α -demethylase, a key enzyme in the biosynthesis of ergosterol; (c) inhibitors of DNA and RNA synthesis; and (d) inhibitors of 1,3- β -glucan synthesis.

Evolution of medical practices with the introduction of new therapies, such as the use of more aggressive chemotherapy or new immunosuppressive drugs, like tumor necrosis factor antagonists, anti-CD52 antibody (alemtuzumab), and interleukin receptor antagonists (basiliximab), contribute to increasing in the incidence of invasive mycoses [1]. The progress achieved in increasing the survival time of patients, in combination with the selection pressure generated by the use of prescribed antifungal preparations for prophylaxis or preventive treatment, also are factors which strengthen the frequency of opportunistic mycotic infections, but also cases of pronounced resistance to applied antimycotic therapy.

Microorganisms develop common mechanisms to counteract the fungicidal and fungistatic effects of antifungal preparations. Currently, it is considered that resistance to drugs of fungi is based on three main mechanisms, namely, (a) reducing the drug accumulation within the fungal cell, (b) reducing the drug affinity towards its target and (c) alteration of metabolism in order to counterbalance the effect of the drug [7].

Adverse effects, often severe, of the antifungal treatment in combination with high rate of multiple resistances of pathogens dictate the necessity of new preparations intended for treatment of invasive infections caused by fungi. Preparations of natural origin are in the center of attention

for both pronounced biological effects and the fact that these are more easily accepted by patients. Biomass of various plants and microorganisms is considered as a raw material for the extraction of compounds with the potential fungicidal and fungistatic effect. *Arthrospira platensis* (*Spirulina*) is a cyanobacterium used extensively as a source of protein, but also of substances with high biological activity, including antiviral, antibacterial and antifungal activity [8–10].

Phenolic extract from *Spirulina platensis* has pronounced influence on the production of structural components of *Aspergillus flavus*. At a concentration 1.15 mg of phenols extracted from 1 g of *Spirulina platensis* biomass the amount of glucosamine in the fungal biomass decreases by 56%. Thus, the biomass and alcoholic extract from *Spirulina* possess antifungal action towards *Aspergillus flavus* [11]. Purified water extracts and concentrates from spirulina showed pronounced antifungal action towards *Penicillium oxalicum* (91% inhibition) and *Fusarium solani* (65% inhibition) [8]. Methanolic extract from dry spirulina biomass possesses antifungal activity towards *Aspergillus flavus* and *Aspergillus niger* [10]. The above mentioned authors stress that the mechanisms of action of spirulina extracts on filamentous fungi are based on inhibiting the synthesis of ergosterol, glucosamine and proteins.

Many biologically active compounds used as drugs exhibit different pharmacological properties and toxic potential, when administered in the form of metal-based compounds [12]. Thus, biomass enriched with various metals could serve as a perspective source in order to obtain efficient preparations for treatment of different diseases, including invasive mycoses.

The aim of the researches presented in this article is to highlight the antifungal properties of extracts obtained from *Spirulina* biomass enriched with metals (Zn, Fe, Cu, Cd, Co și Cr) towards some representatives of the genera *Aspergillus*, *Penicillium*, *Fusarium* și *Mucor*.

Material and methods

Aspergillus fumigatus CNM-FA-02, *Mucor vulgaris* CNMN-FD-07, *Penicillium expansum* CNMN-FD-05 from National Collection of Microorganisms of the Institute of Microbiology and Biotechnology of the Academy of Sciences of Moldova, have been used as reference strains. Two strains of fungi that represent spontaneous flora, which were isolated from soil and identified as *Fusarium solani* and *Fusarium oxysporum* were also included in this study. Fungi were grown on malt agar medium at a temperature of 30 °C.

Sensitivity to the action of extracts from spirulina biomass was determined by the agar-well diffusion method. There was poured agar medium populated by test-culture in Petri dishes with a diameter of 100 mm. Culture (in the form of spores) was introduced into the medium when it

has reached a temperature of 65-70 °C. The inoculum had a concentration of about 20 mln spores to 1 ml. Wells were drilled into the agar using sterile drill. The diameter of the wells was 8 mm. There were introduced equal volumes of the standard solution and the tested solutions in wells. Petri dishes were left at room temperature for 2 hours, after which were incubated at 30±1°C for 96 hours. The inhibition zone diameter of test-microorganisms growth has been measured using caliper with accuracy of 0.1 mm.

There were used two types of extracts from biomass of *Arthrospira platensis* CNMN-CB-11 as preparations with antifungal effect. The cyanobacterium was grown on nutrient medium SP -1 with added metal compounds (Zn(II), Fe(III), Cu(II), Cd(II), Co(II) și Cr(III)). The first type of extracts (EE) has been obtained by extraction with ethyl alcohol of 96% from biomass (2 parts of alcohol volume to 1 part biomass 100 mg/ml) at room temperature for 2 hours on the mechanical stirrer. The second type of extracts (HE) was obtained by extraction in hot water (90°C) for 1 hour, observing the same report – 2 parts of purified water volume and 1 part biomass 100 mg/ml. The extracts have been standardized after the dry substance so that 1 ml of extract contains 10 mg dry substance. Content of metals in the extracts was the following: EE_{Zn} – 0,15%; HE_{Zn} – 0,38%; EE_{Fe} – 0,09%; HE_{Fe} – 0,08%; EE_{Cu} – 0,35%; HE_{Cu} – 0,42%; EE_{Cd} – 0,09%; HE_{Cd} – 0,03%; EE_{Co} – 0,09%; HE_{Co} – 0,09%; EE_{Cr} – 0,11%; HE_{Cr} – 0,15%. Corresponding extracts from standard biomass were taken as control: EE-ethanolic extract, HE-hydric extract. There was also taken naftifine hydrochloride (NH), solution, 10 mg/ml, as reference antifungal compound.

Antifungal effect of preparations had been also appreciated after the activity of lactate dehydrogenase (LDH). The release of this cytoplasmic enzyme, which shows instability of cell membranes, was determined by applying the procedure described by Arokiyaraj and coauthors with application to the strains of filamentous fungi [13]. Summarily, the determination has been made in the following manner: the strains were grown through submerged cultivation on Sabouraud medium. At the transition of fungi into stationary phase of the vital cycle, biomass was separated from the nutrient medium, washed and resuspended in purified water, to which in the experimental variants were added extracts from *Spirulina* (200 mg dry substance/L). Incubation was carried out at room temperature for 4 hours. 100 μL of supernatant of each control cultures and those treated with extracts from spirulina, were added to the reaction mixture containing 0.5 ml pyruvate of 100 mM, 5 mg NADH in 20 ml buffer solution of potassium phosphate of 500 mM, pH 7.5. Absorbance (A) was read for 5 min at intervals of 30 seconds at the wavelength of 340 nm at the spectrophotometer UV-VIS PG Instrument T-80. LDH activity in international units (U/L), which expresses the amount of enzyme which reduces 1 μM of NAD per minute, was calculated according to the formula:

$$U/L = \frac{\Delta A / \min \times TV \times 1000}{d} \times d \times \varepsilon \times SV$$

$$U/L = \frac{\Delta A / \min \times TV \times 1000}{d} \times d \times \varepsilon \times SV$$

Where $\Delta A / \min$ represents the relative change of absorbance at 340 nm, TV is the total volume of reaction, 1000 is the passage coefficient from U/ml to U/L, d is the luminous flux in cm, ε represents the molar extinction coefficient, SV is the volume of sample in ml [14].

All experiments were performed in three replicates. There was calculated the average value and the standard deviation. The veracity of the differences between the experimental and control variants was assessed on the basis of Student criterion.

Results and discussion

The results obtained in case of testing the antifungal action of extracts from spirulina standard biomass and containing metals through diffusion method are presented in Table 1. It includes the diameters of the inhibition zones of fungal growth and P values only for cases excluded the null hypothesis and indicates the difference between the variants in the direction of increasing the effects of the tested preparations. Ethanolic extract from standard biomass of

Spirulina had an inhibitory effect on the growth of only one of the tested strains – *Aspergillus fumigatus* CNM-FA-02. Extracts from other types of biomass also had an inhibitory effect on the growth of *Aspergillus fumigatus*. In the case of extracts from spirulina biomass containing copper and cadmium antifungal effect is more pronounced than in the case of the ethanolic extract from standard biomass. The diameters of the inhibition zones are higher ($P=0.005$) than in case of standard extract. However, even these two extracts remain significantly weaker than antifungal preparation of reference naftifine hydrochloride. While the growth of other tested fungal strains was not inhibited by the ethanolic extracts from standard biomass, the extracts from biomass with metals have manifested an antifungal effect of different intensity against all investigated fungi. Antifungal effect towards *Penicillium expansum* CNMN-FD-05, *Fusarium oxysporum* and *Fusarium solani* of extracts from spirulina containing metals is small compared to that of naftifine hydrochloride in all the experimental variants. There have been highlighted the results recorded for the strain *Mucor vulgaris* CNMN-FD-07. In this case 4 of 6 extracts from biomass with metals showed a more pronounced antifungal effect than the reference substance naftifine hydrochloride. With the exception of the extract from *Spirulina* biomass containing copper, which produces no inhibition effect, and from biomass containing iron,

Table 1

Antifungal effect of extracts from *Spirulina* biomass with metals towards some strains of filamentous fungi

Extract/Substance	Diameter of inhibition zone, mm				
	<i>Aspergillus fumigatus</i> CNM-FA-02,	<i>Mucor vulgaris</i> CNMN-FD-07	<i>Penicillium expansum</i> CNMN-FD-05	<i>Fusarium oxysporum</i>	<i>Fusarium solani</i>
EE _{Zn}	11,66±0,58	14,23±1,12 $P_2=0,019^{**}$	10,63±0,72	10,73±0,64	9,70±0,31
EE _{Fe}	0	10,27±0,67	10,37±0,57	11,70±0,79	11,40±0,62
EE _{Cu}	19,33±0,81 $P_1=0,005^*$	0	10,20±0,91	11,67±0,60	10,03±0,35
EE _{Cd}	20,33±1,00 $P_1=0,005$	13,63±0,74 $P_2=0,013$	19,13±1,15	14,23±0,59	13,23±0,89
EE _{Co}	11,2±0,79	23,30±1,05 $P_2=0,001$	11,37±0,25	11,70±0,30	11,40±0,90
EE _{Cr}	15,87±0,83 $P_1=0,008$	18,43±0,61 $P_2=0,003$	16,70±0,55	12,67±1,52	10,83±0,56
EE	12,76±0,74	0	0	0	0
HE _{Zn}	0	0	0	0	0
HH _{Fe}	0	0	0	10,0±0,26	0
HE _{Cu}	0	0	0	0	0
HE _{Cd}	28,67±0,58	0	27,33±0,64	16,67±0,64	21,6±0,53
HE _{Co}	0	19,2±0,72	16,07±0,90	29,67±0,577 $P_2=0,001$	0
HE _{Cr}	15,1±0,17	0	21,33±0,58	24,67±0,57	0
HH	0	0	0	0	0
Naftifine hydrochloride	34,5±1,08	11,63±0,35	34,67±0,31	24,17±0,76	31,10±0,85

P_1^* - veracity of differences between the variants EE_{Me} and variant EE.

P_2^{**} - veracity of differences between the variants with extracts and naftifine hydrochloride.

inhibition zones in experimental variants were more extensive compared to those obtained in the case of the reference substance ($0.003 \leq P \leq 0.019$).

Water extracts from *Spirulina* biomass were less active as preparations with antifungal activity. Extract from standard biomass has not inhibited the growth of studied filamentous fungi. The same thing can be said about water extracts from *Spirulina* biomass containing zinc and copper – inhibition zones of fungal growth have not been certified. Water extract with iron showed weak antifungal action only towards strain isolated from the spontaneous edaphic microflora – *Fusarium oxysporum*.

Experimental variant HE_{Cd} containing cadmium showed antifungal effect towards 4 strains of fungi (with the exception of *Mucor vulgaris* CNMN-FD-07). Inhibition zones of growth of fungi *Penicillium expansum* CNMN-FD-05 and *Aspergillus fumigatus* CNM-FA-02 were similar to naftifine hydrochloride zones, but nonetheless smaller in diameter. Water extract from *Spirulina* biomass containing cobalt has inhibited the growth of three strains of fungi – *Mucor vulgaris* CNMN-FD-07, *Penicillium expansum* CNMN-FD-05 and *Fusarium oxysporum*. In the last case, the antifungal effect of the extract was more pronounced than that of naftifine hydrochloride, the inhibition zone being higher by 22.76%.

Hydric extract with chromium was manifested by growth inhibition of *Aspergillus fumigatus* CNM-FA-02, *Penicillium expansum* CNMN-FD-05 and *Fusarium oxysporum*. In this case, inhibition zone of growth of *Fusarium oxysporum* is equal to that measured in the variant with naftifine hydrochloride.

Then, it was determined the level of release into the medium of the enzyme lactate dehydrogenase in experimental variants in which antifungal activity was detected. In the case of the integrity of cell sheaths it remains seized in the cytoplasm of cells and only in the event of disturbing the structure and functions of the membrane and cell wall the enzyme is released into the medium. Increasing the activ-

ity of lactate dehydrogenase is an indicator of toxicity of substances, which have acted upon the studied cultures. Figure 1 represents the activity of extracellular lactate dehydrogenase at the action of spirulina extracts on *Aspergillus fumigatus* CNM-FA-02.

Under normal conditions the activity of extracellular LDH from *Aspergillus fumigatus* CNM-FA-02 constitutes 34 international units per liter. The same level of activity can be also observed in supernatant of culture treated with hydric extract obtained from *Spirulina* standard biomass. In the case of the ethanolic extract from standard biomass we can observe an increase of LDH activity by 23.5% compared to the control, which is also associated with the described above inhibitory effect of culture growth. Extracts from biomass containing cadmium, both ethanolic and hydric one, leading to duplication of LDH activity, the values are practically equal with those obtained for naftifine hydrochloride. In the case of extracts from biomass containing copper, cobalt and chromium, the effect of disturbance of the permeability of cell sheaths is much higher when applying the ethanolic extracts than water ones, but the effect is present in all these variants. In all these cases, it has been also observed the inhibition of fungi growth under the influence of mentioned extracts. The results presented in this figure emphasize the ethanolic extract obtained from *Spirulina* biomass containing zinc. Though, it was previously described moderate effect of inhibition of *Aspergillus* growth to the action of this extract, LDH activity is quite low – 14.7% compared to the control.

Figure 2 represents the activity of extracellular lactate dehydrogenase to the action of spirulina extracts on *Mucor vulgaris* CNMN-FD-07 and *Penicillium expansum* CNMN-FD-05.

A very pronounced increase of LDH activity at *Mucor vulgaris* CNMN-FD-07 was registered in the experimental variants with ethanolic extract from biomass containing cadmium, cobalt, chromium, the values being 2.7-3.2 times higher than in the case of the control. Moreover, these ex-

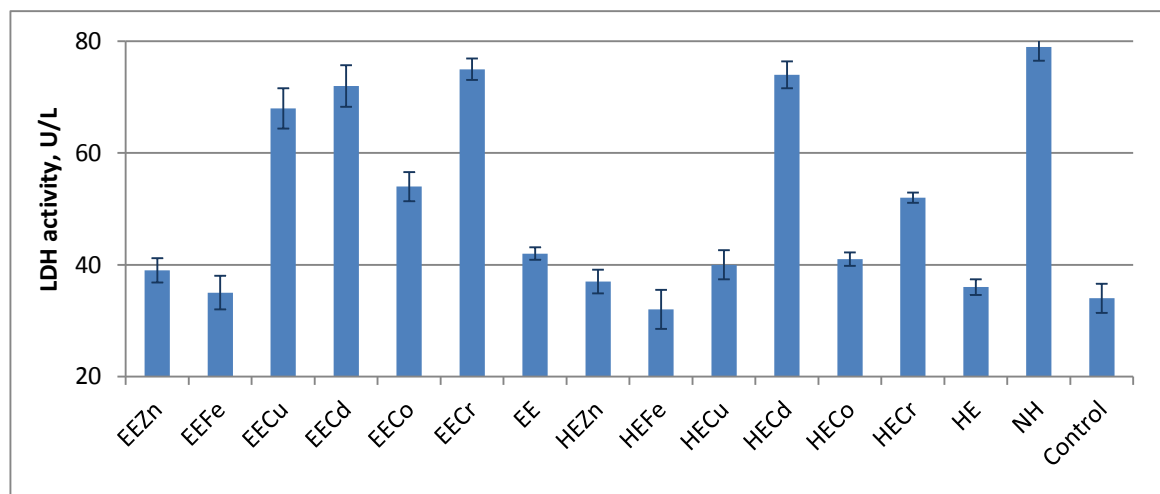


Fig. 1. Lactate dehydrogenase activity released by the culture *Aspergillus fumigatus* CNM-FA-02 under the action of spirulina extracts (NH - naftifine hydrochloride).

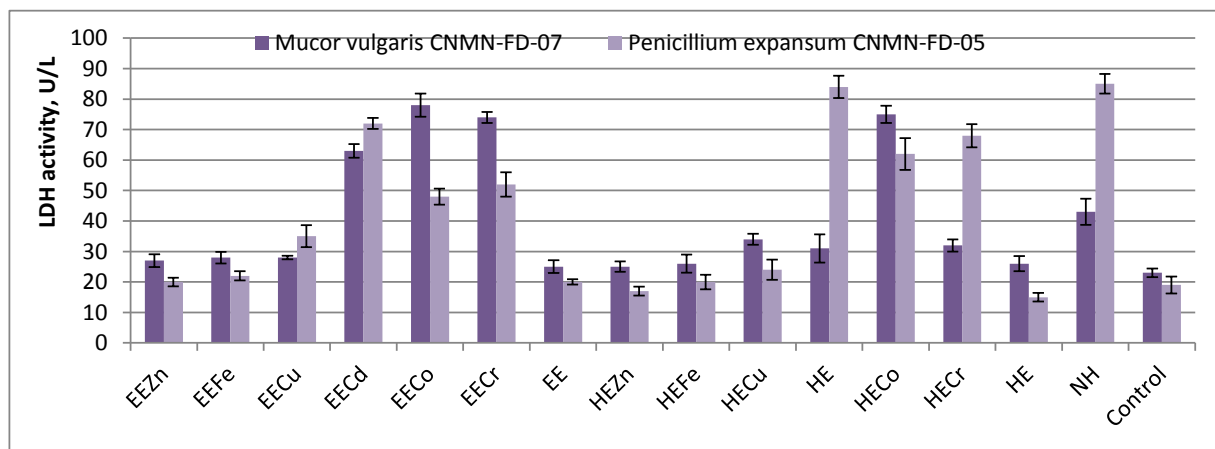


Fig. 2. Lactate dehydrogenase activity released by the culture *Mucor vulgaris* CNMN-FD-07 and *Penicillium expansum* CNMN-FD-05 under the action of spirulina extracts (NH-naftifine hydrochloride).

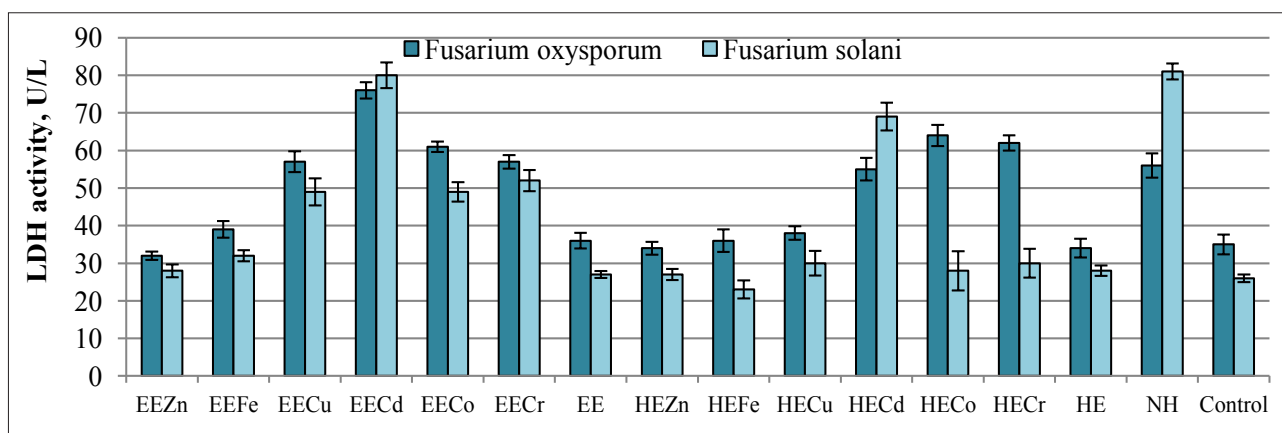


Fig. 3. Lactate dehydrogenase activity released by the culture *Fusarium oxysporum* and *Fusarium solani*

ceed the LDH activity by 1.47-1.81 times in the variant with naftifine hydrochloride. Of all the water extracts, a pronounced toxic effect was registered only for the variant containing cobalt, in which LDH activity is 3.26 times higher than in the case of control and 1.74 times higher than in the case of application of naftifine hydrochloride.

Water and ethanolic extracts from biomass containing cadmium, cobalt and chromium have manifested antifungal effects towards *Penicillium expansum* CNMN-FD-05. In the case of applying the ethanolic extracts, the activity of LDH released into the extracellular medium is 2.7-3.8 times higher than control, but none of the tested variants showed higher values than those obtained from the treatment of culture with naftifine hydrochloride. Ethanolic extract containing copper has also produced the release of LDH in the medium, thereby increasing its activity by 1.84 times. Water extracts containing cobalt, chromium and cadmium also led to the disruption of membrane permeability of *Penicillium*, expressed through intense elimination of LDH. In the case of water extract with cadmium, LDH activity is at the level of the sample with naftifine hydrochloride. And for these two strains, although ethanolic extracts containing iron and zinc have inhibited growth

on agar medium, especially *Mucor*, where inhibition was at the level of naftifine hydrochloride, treating the fungal biomass does not lead to increase of LDH activity.

Figure 3 represents the activity of extracellular lactate dehydrogenase to the action of spirulina extracts on the strains isolated from spontaneous microflora *Fusarium oxysporum* and *Fusarium solani*.

We can see from the figure, that in the case of these two fungi strains, the ethanolic extracts containing copper, cadmium, cobalt and chromium possess higher toxic potential, which causes the active elimination of LDH in extracellular medium. All the experimental variants showed values of LDH activity of 2.5-3.3 times higher than control. Ethanolic extract containing cadmium has a higher toxicity towards *Fusarium oxysporum* than naftifine hydrochloride, LDH activity released by the culture being 1.36 times higher. Water extracts from these types of biomass have led to an increase in LDH activity, but only in the case of *Fusarium oxysporum*. Only water extract from biomass containing cadmium had toxic effect against strain *Fusarium solani*. Ethanolic extracts from biomass containing iron and zinc produced inhibition of *Fusarium* growth, but excessive elimination of LDH under their influence has not been observed.

Conclusions

Ethanol and hydric extracts from biotechnologically obtained biomass of *Arthrospira platensis*, under conditions of bioaccumulation of metals, are characterized by antifungal activity of different intensity against filamentous fungi taken in study, while the extracts obtained from standard biomass are lacking this capacity (with the exception of the ethanol extract towards *Aspergillus fumigatus* CNM-FA-02). The majority of ethanol extracts are toxic to fungal cultures, which is expressed both by the development of the inhibition zones of growth at the application of agar diffusion method and by the release of lactate dehydrogenase in the extracellular medium. Ethanol extracts from *Spirulina* biomass containing zinc, cobalt, cadmium and chromium have been manifested as preparations with antifungal properties towards strain *Mucor vulgaris* CNMN-FD-07, their action being superior to naftifine hydrochloride.

There were highlighted water extracts obtained from biomass containing cadmium, cobalt and chromium, that showed growth inhibitory effect on 3-4 tested strains. Water extracts containing cobalt and chromium showed pronounced antifungal effect towards *Fusarium oxysporum*, equal (HE_{Cr}) or higher (HE_{Co}) than naftifine hydrochloride.

In the case of treating the fungal cultures with *Spirulina* extracts containing copper, cadmium, chromium and cobalt, the growth inhibitory effect of the mycelium is associated with the release of lactate dehydrogenase in the extracellular medium, which denotes disruption of permeability of cell sheaths. In the case of biomass extracts containing iron or zinc, inhibition of growth is not followed by the release of LDH, which denotes a different mechanism of action that will be researched further.

Extracts from *Spirulina* biomass with metals linked in structure of organic compounds are promising in order to obtain preparations with antifungal action towards causative agents of invasive mycoses.

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Surveillance of other beta-lactam antibacterials in Emergency Medicine Institute

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Abstract

Background: The use of other beta-lactam antibacterials in the Emergency Medicine Institute (EMI) recorded in the medium 60%, while around the world in hospitals only 15-20% on average, from all yearly antibiotics consumption. That argument represents an important scientific and practical interest for evaluation of other beta-lactam antibacterials.

Material and methods: For this study we used the data of a six-year (2009-2014) period in the EMI and their subdivisions with main consumption of antibiotics which show dynamics of the use of other beta-lactam antibacterials in grams and value indexes.

Results: In EMI, during the evaluated period other beta-lactam antibacterials recorded a stable consumption (Defined Daily Doses, DDD) from 270.8 to 272.6 DDD/1000 or 58.74% of the total in 2014, with an increase of 0.67% and a decrement in septic surgery and orthotrauma (SSOT) departments from 238.6 to 231.54 DDD/1000 or by 2.96%. A sharp decrease registered in intensive care (IC) departments from 1416.54 to 636.78 DDD/1000 or by 55.05%. In the end of the evaluated period IC departments recorded 24861.5 lei per DDD/1000 that was 7.18 times more than the cost of 3460.46 lei registered in SSOT departments and respectively 4.53 times higher than the cost of 5489.7 lei recorded per DDD/1000 in the entire EMI.

Conclusions: We find that in the evaluated period in EMI, the consumption of other beta-lactam antibacterials recorded in medium the similar yearly data, while the same data in some European and Australian hospitals are on average respectively by 2.18 and 1.32 times less.

Key words: other beta-lactam antibacterials, defined daily dose, consumption, rational use, hospitals.

Introduction

Increment of antibiotic resistance and consumption is one of the most serious global threats to the treatment of infectious diseases [1, 2, 3, 4, 5]. In addition as a consequence it results in significant increases in costs and toxicity of newly appeared drugs, antibiotic resistance complicates the quality of therapeutic treatment. Countries and hospitals with the fewest control in antibiotic prescribing have the greatest frequency of resistant organisms, [6] which suggests a causal connection and necessity of antibiotics consumption evaluation. In The United States, 160 million antibiotic prescriptions are written annually for humans; these figures equate to 30 prescriptions and 4.1 kg of antibiotics per 100 persons per year. Such industrialized nations as France, Australia, The United States, Canada, Italy and the United Kingdom have the highest rates of oral antimicrobial prescriptions, ranging from 33 to 16 defined daily doses per 1000 population per day [7, 8]. All around the world in hospitals the use of other beta-lactam antibacterials recorded approximately 15-20%, while in EMI 50-60% of all antibiotics consumption. That situation determined higher attention for this group of anti-infectives for systemic use in medicine [9, 10, 11] including surveillance, stringent use control and rational prescription, supporting the importance of antimicrobial consumption on resistance [12].

The primary aim of the study was to evaluate institutional representative data on other beta-lactam antibacterials utilization, in accordance with the World Health Organization (WHO) requirements, directed to determine the value of Defined Daily Doses (DDD) per 1000 Occupied-Bed Days (DDD/1000) and value cost in the dynamics per total institution and most important departments [13].

Material and methods

For this study we used the data of a six-year (2010-2014) period DDD/1000 consumption of other beta-lactam and penicillin antibiotics in EMI (Emergency Medicine Institute) and their main subdivisions intensive care departments (ICD – reanimation, intensive therapy and intensive neurological “stroke” departments) and SSOTD (septic surgical and septic orthotraumatology departments) [14] which show the dynamics of consumption of anti-infectives for systemic use drugs as classified by Anatomical Therapeutic Chemical (ATC) classification system of World Health Organization (WHO) indicated in grams and value indexes. Statistical, analytical, mathematical, comparative, logical and descriptive were used as the methods of study.

Results and discussion

For determining the number of DDD/1000 we used data about total annual consumption of other beta-lactam antibacterials and the statistics data concerning the number of treated patients (only patients with health insurance and other free treated by the state categories of citizens). The total number of occupied bed/days in the institution was 188762 in 2009, 191556 in 2010, 186246 in 2011, 199816 in 2012, 193019 in 2013 and 187558 in 2014 and respectively for the evaluated departments of EMI: reanimation department (2009 = 3990; 2010 = 6551; 2011 = 6985; 2012 = 9051; 2013 = 7384; 2014 = 7361), intensive therapy department (2010 = 2922; 2011 = 3327; 2012 = 3239; 2013 = 3407; 2014 = 3388), intensive neurological “stroke” department (2013 = 2553; 2014 = 4193), septic surgical department (2009 = 14030; 2010 = 14212; 2011 = 12875; 2012 = 12372; 2013 =

12464; 2014 = 12104), septic orthotraumatology department (2009 = 10664; 2010 = 10017; 2011 = 9540; 2012 = 10178; 2013 = 9701; 2014 = 9535) [15, 16, 17, 18].

Consumption of other beta-lactam antibacterials in EMI is characterized by the use of parenteral (P) and enteral (E) forms of many subgroups with the respective nomenclature of antibiotics as following: the first-generation of cephalosporins (Cefalexinum DDD 2.0E, Cefazolinum DDD 3.0P), the second-generation of cephalosporins (Cefuroximum DDD 0.5E, 3.0P, Cefaclorum DDD 1.0E), the third-generation of cephalosporins (Cefotaximum DDD 4.0P, Ceftazidimum DDD 4.0P, Ceftriaxonum 2.0P, cefixim DDD 0.4E, Cefoperazonum DDD 4.0P, Cefoperazonum + culbactamum DDD 4.0P) and carbapenems (Meropenemum DDD 2.0P, Imipenemum+cilastatinum DDD 2.0P). Total other beta-lactam antibacterials consumption in DDD/1000 during 2009-2014 is shown in figure 1.

From figure 1, it could be observed a total decrease of other beta-lactam antibacterials consumption for all departments. According to the annual medium consumption of all departments of 2701.58 DDD/1000 could be placed as following: first – reanimation department with 970.38 DDD/1000 or 35.92% and a decrease from 1416.54 to 886.7 DDD/1000 or by 37.34%, second – intensive therapy department with 794.95 DDD/1000 or 29.43% and a de-

crease from 974.67 in 2010 to 597.7 or by 38.68%, third - intensive neurological «stroke» department with 467.76 DDD/1000 or 17.31% and a decrease from 509.6 in 2013 to 425.95 or by 16.42%, fourth - septic surgical department with 237.92 DDD/1000 or 8.81% and a decrease from 310.05 to 187 DDD/1000 and septic orthotraumatology department with 230.57 DDD/1000 or 8.53% and an increase from 167.1 to 276.14 DDD/1000 or by 65.25% on the position number five. In figure 2 the total other beta-lactam antibacterials consumption of parenteral forms in DDD/1000 during 2010-2014 is shown.

In figure 2 parenteral forms of other beta-lactam antibacterials consumption is presented. As it could be observed from figure 1 and 2 the consumption of parenteral forms of other beta-lactam antibacterials in the mean are similar with the total results.

In figure 3 totals DDD/1000 of other beta-lactam antibacterials (enteral forms) consumption during 2009-2014 are shown.

The data from figure 3 shows that in the evaluated period enteral forms of other beta-lactam antibacterials recorded a significant increment from 0.93 to 61.25 DDD/1000 or by 65.86 times in septic orthotraumatology department and from 2.75 to 10.08 DDD/1000 or by 3.67 times in septic surgical department. Other departments

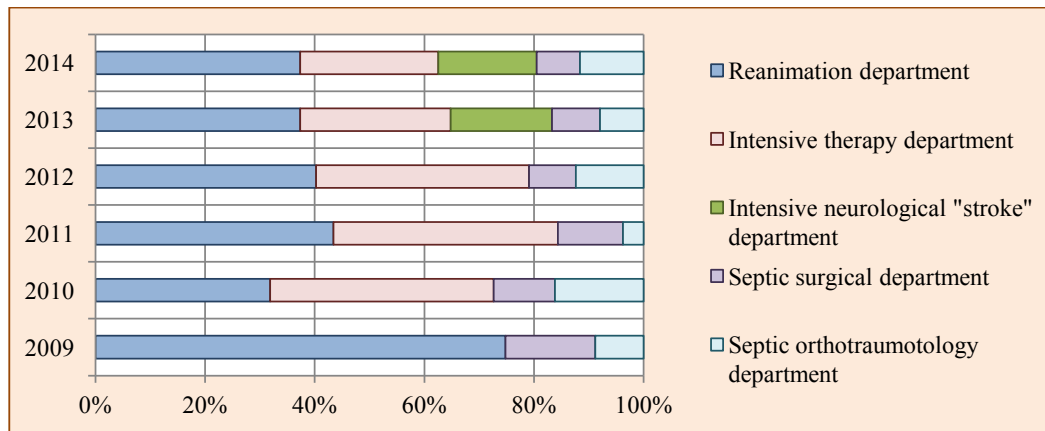


Fig. 1. Total other beta-lactam antibacterials consumption in DDD/1000 during 2009-2014.

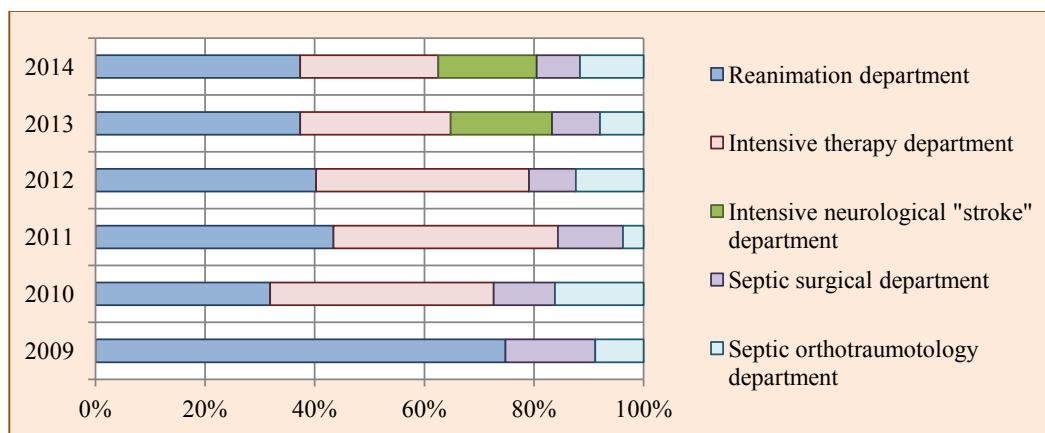


Fig. 2. Total other beta-lactam antibacterials consumption in DDD/1000 (parenteral forms).

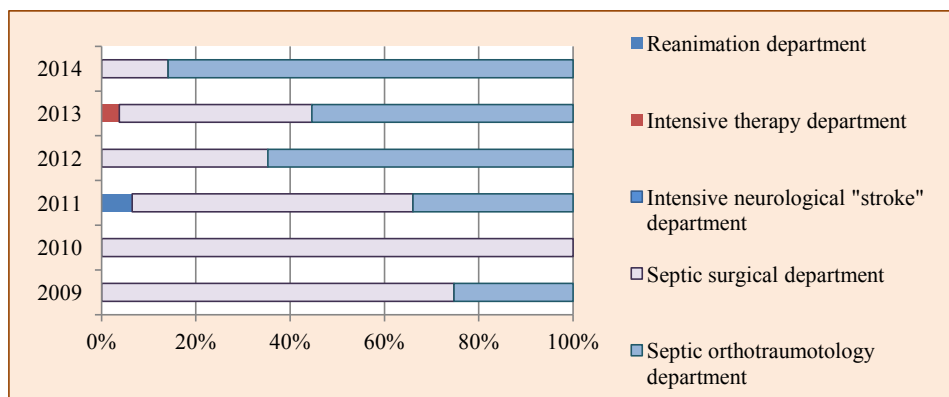


Fig. 3. Total other beta-lactam antibacterials consumption in DDD/1000 (enteral forms).

registered an occasional or didn't record any consumption of this group of antibiotics.

Taking into consideration the situation that in most scientific journals the published data about drugs consumption include the use of them in all intense care hospital unites, we determined the medium consumption of DDD/1000 separately for ICD and SSOTD (septic surgical and septic orthotraumatology departments) of EMI. To determine the medium consumption of DDD/1000 we counted total of DDD/1000 separately for ICD and SSOTD and divided by the number of those departments (3 and respectively 2). The results are shown in table 1.

The data in table 1 shows that in the evaluated period total consumption in ICD departament of other beta-lactam antibacterials decreased from 1416.54 to 636.8 DDD/1000 or by 55.05% and respectively in SSOTD from 238.9 to 231.54 DDD/1000 or by 3.08%. Consumption in ICD departments in 2014 was by 2.75 (636.8:231.54) times more than in SSOTD departments. Total institutional parenteral forms recorded a decrease from 268.6 to 239.3 DDD/1000 or by 10.91% and vice versa enteral forms a significant increase from 2.2 to 25.3 DDD/1000 or by 11.5 times. In table 2 total records of parenteral and enteral forms consumption in EMI are shown.

From table 2 it could be observed that during the evalu-

ated period other beta-lactam antibacterials in EMI recorded a stable consumption from 270.8 to 272.6 DDD/1000 with an increase of 0.67% that in 2014 represents a share of 58.74% of the total. In large acute international public hospitals and other international hospitals, it was registered an increase from 191 to 206.31 DDD/1000 or by 7.91% that in 2014 represented a share of 22.01% of the total of 936.31 DDD/1000. In some hospitals of European countries consumption of this group of drugs recorded a decrease from 144.5 to 125 DDD/1000 or by 13.40% that in 2013 represented a share of 17.60% from the total. In a Single University Hospital a medium consumption in the period from 2001 to 2012 recorded 348.2 DDD/1000 or a share of 54% of the total 644.6 DDD/1000. In French hospitals in 2007 consumption represented 67.7 DDD/1000 or 12.14% from the total of 557.7 DDD/1000 in 27 public teaching hospitals, 36.3 DDD/1000 or 9.77% from the total of 371.5 DDD/1000 in 165 non – teaching public hospitals and 67.3 DDD/1000 or 16.14% from the total of 416.9 DDD/1000 in 158 private hospitals [21]. The same data in 130 US hospitals in 2002-2003 represented 80.3 DDD/1000 or 10.14% from total 792 DDD/1000 [22].

The value cost of other beta-lactam antibacterials use per DDD/1000 in lei is presented in figure 4.

As it could be seen from figure 4, during the evaluated

Table 1

Other beta-lactam antibacterials (parenteral and enteral forms) consumption in DDD/10

Department	Administration	2009	2010	2011	2012	2013	2014
ICD	Parenteral	1416.54	868.73	871.39	812.51	765.97	636.8
	Enteral			1.43		1.47	
	Total	1416.54	868.73	872.1	812.51	766.33	636.78
SSOTD	Parenteral	236.76	326.54	151.47	207.8	212.54	195.87
	Enteral	1.84	0.71	10.25	7.22	18.81	35.67
	Total	238.6	327.25	161.72	215.02	231.35	231.54
Total EMI	Parenteral	268.6	257.6	256.1	213	256.6	239.3
	Enteral	2.2	3.6	16.7	12.4	14.1	25.3
	Total	270.8	261.2	272.7	225.6	270.7	272.6

Table 2

Other beta-lactam antibacterials consumption of DDD/1000 in EMI and some international hospitals

Programs/institutions	2009	2010	2011	2012	2013	2014
Emergency Medicine Institute	270.8	261.2	272.7	225.6	270.7	272.6
Total	662.4	558.2	622.1	542.4	546.9	464.1
Percentage	40.88%	46.79%	43.84%	41.59%	49.5%	58.74%
Large acute Australian pub. hospitals [19]	191	193.1	222	178.4	186.9	206.11
Total	931.8	933.7	946.5	931.6	943.4	936.31
Percentage	20.49%	20.68%	23.45%	19.15%	19.77	22.01%
	2012		2001 - 2012 - 2013		2013	
Single University Hospital [20]			348.2			
NAUSP; SAAUSP [19]			203;186			
DANMAP; SWEDRES; NETHMAP	(192+97):2=144.5				125	
Total	(931+609):2=770		631;945;943		712	
Percentage	18.77%		55%;27%; 22%;29%		17.6%	

period the main value cost of DDD/1000 recorded reanimation department from 40128.3 lei to 33981.3 lei or a decrease by 15.32%, consequently the second position holds intensive therapy department from 35143.5 lei in 2010 to 29271.2 lei or a decrease by 16.71% and thirdly the intensive neurological «stroke» department from 11695 lei in 2013 to 11332 lei or a decrease by 3.10%, followed by septic surgical department from 5495.4 lei to 3330.55 lei or a decrease by 39.39% and the last position was held by septic orthotraumatology department from 3177.24 to 3590.36 lei or an increment by 13%.

In figure 5 the total value cost of other beta-lactam antibacterials in DDD/1000 (parenteral forms) is presented.

The cost of parenteral other beta-lactam antibacterials in DDD/1000 for all departments remains approximately the same comparatively with the total consumption because of low cost of enteral forms for DDD/1000. In figure 6 the value cost of DDD/1000 in lei of other beta-lactam antibacterials enteral forms is shown.

Presented data in chart 6 demonstrates that from the total departments annual costs could be placed as following: the first septic orthotraumatology department with the cost per DDD/1000 from 3.02 lei to 329.52 lei and the second septic surgical department with the cost from 46.73 lei to 72.45 lei per DDD/1000. Other departments because of the lack or very low consumption didn't record at all, or recorded an episodic price per DDD/1000.

To determine the medium cost of DDD/1000 of other beta-lactam antibacterials was counted the total cost of DDD/1000 separately for ICD and SSOTD and divided by the number of those departments (3 and respectively 2).

As it could be seen from table 3 in the evaluated period total medium cost of DDD/1000 for other beta-lactam antibacterials recorded a decrease in ICD departments from 40128.3 lei to 24861.5 lei or by 38.05% and consequently, in SSOTD from 4336.32 lei to 3460.46 lei or by 20.20%. Medium cost of DDD/1000 in SSOTD departments was less than in ICD departments by 8.21 times in 2009 and re-

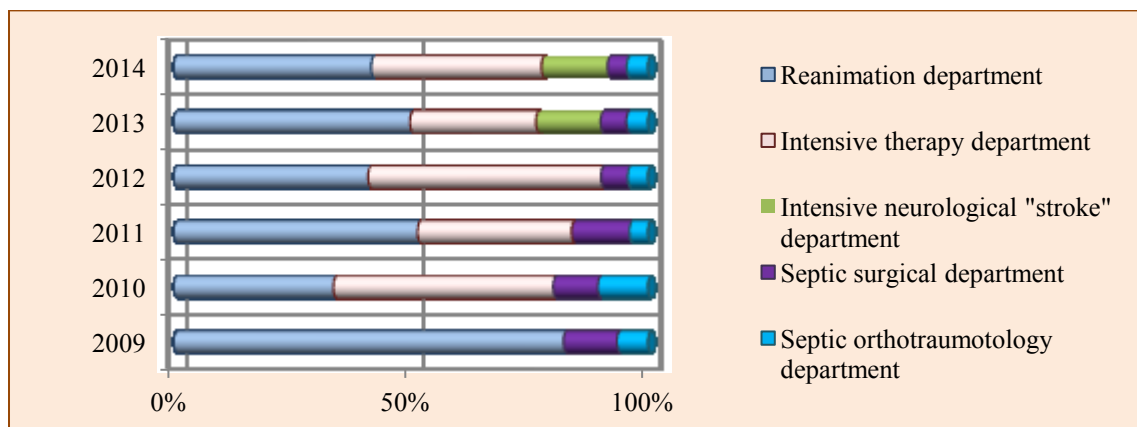


Fig. 4. Total value cost of other beta-lactam antibacterials per DDD/1000 in lei.

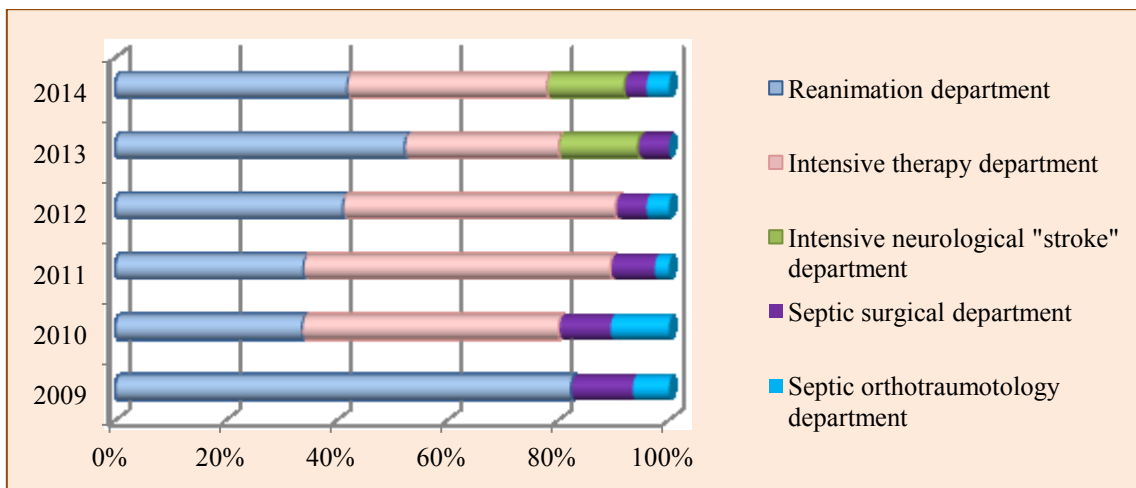


Fig. 5. Value cost of other beta-lactam antibacterials in DDD/1000 of parenteral forms in lei.

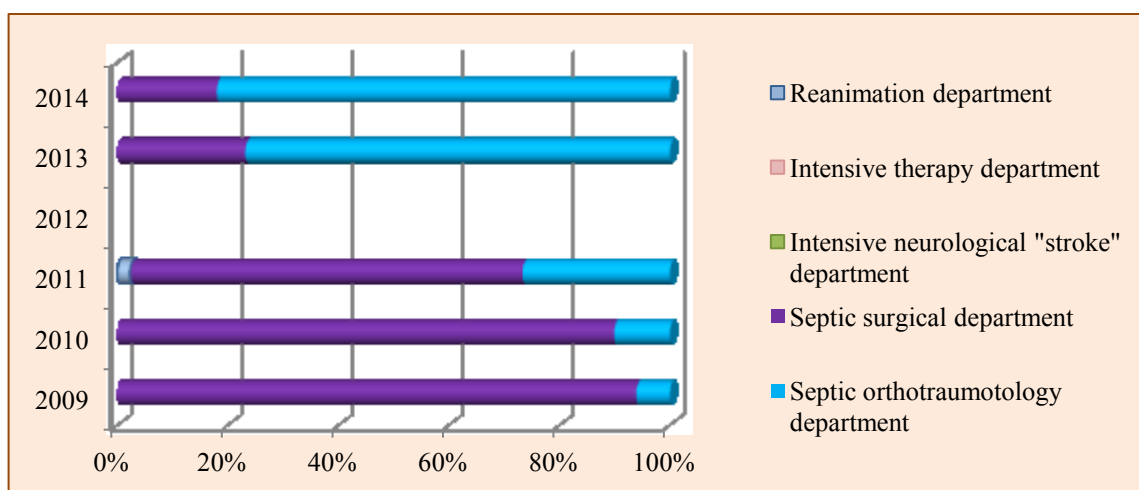


Fig. 6. Value cost of other beta-lactam antibacterials in DDD/1000 (enteral forms) in lei.

Table 3

Medium cost of DDD/1000 in lei of other beta-lactam antibacterials (parenteral and enteral forms) in EMI

Department	Structure of consumption	2009	2010	2011	2012	2013	2014
ICD	Parenteral	40128.3	30354.97	22060.76	26212.93	25760.17	24861.52
	Enteral			1.43		5.83	
	Total	40128.3	30355	22062.2	26212.9	25762.1	24861.5
SSOTD	Parenteral	4311.445	7541.72	4194	2794.39	2290.455	3259.47
	Enteral	24.88	14.31	30.27	67.29	137.86	200.99
	Total	4336.32	7556.03	4224.27	2861.68	4259.34	3460.46
Total EMI	Parenteral	4888.31	5873.17	4942.22	4473.53	5278.44	5368.52
	Enteral	17.18	26.03	103.44	136.74	205.86	121.19
	Total	4905.49	5899.2	5045.66	4610.27	5484.3	5489.7

spectively by 7.18 times in 2014. At the same time, the total institutional cost of DDD/1000 increased from 4905.49 in 2009 to 5489.7 DDD/1000 in 2014 or by 11.91% and was less than medium cost in ICD departments by 4.53 times.

Conclusions

1. In EMI, during the evaluated period the use of other beta-lactam antibacterials recorded a stable consumption in a medium of 272 DDD/1000 in comparison with 125 DDD/1000 in some European hospitals as all as of 206.31 DDD/1000 in Australian acute public hospitals or respectively by 2.18 and 1.32 times less.

2. As to the annual medium consumption of 2701.58 DDD/1000 of all departments places are distributed as following: the first – reanimation department with 970.38 DDD/1000 or 35.92%, the second – intensive therapy department with 794.95 DDD/1000 or 29.43, the third – intensive neurological “stroke” department with 467.76 DDD/1000 or 17.31%, the fourth – septic surgical department with 237.92 DDD/1000 or 8.81% and a decrease from 310.05 to 187 DDD/1000 and septic orthotraumatology department with 230.57 DDD/1000 or 8.53% on the fifth position.

3. Total consumption in ICD departmentals of other beta-lactam antibacterials decreased from 1416.54 to 636.8 DDD/1000 or by 55.05% and respectively in SSOTD from 238.9 to 231.54 DDD/1000 or by only 3.08%. Consumption in ICD departments in 2014 was 2.75 times more than in SSOTD departments.

4. Total medium cost of DDD/1000 for other beta-lactam antibacterials recorded a decrease in ICD departments from 40128.3 to 24861.5 lei or by 38.05% and consequently in SSOTD from 4336.32 to 3460.46 or by 20.20%. At the same time, the total institutional cost of DDD/1000 increased from 4905.49 in 2009 to 5489.7 DDD/1000 in 2014 or by 11.91% and was less than medium cost in ICD departments by 4.53 times.

5. The findings of this study could serve a significant support for hospitals to compare the data concerning antimycotics consumption with the international health care institutions and to optimize in planning necessities as well as to improve rational use of other beta-lactam antibacterials.

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Contemporary profile of the patient with acute pelvic inflammatory disease

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Abstract

Background: The aim of our research included identifying the medical and social determinants that favor the development of acute pelvic inflammatory disease (APID), and assessing the contemporary profile of the APID patient while improving the process of diagnostics and management for this disease.

Material and methods: There were analyzed 644 clinical cases of patients that were selected according to the CDC criteria regarding APID, who were hospitalized in the Department of Septic Gynecology at the Municipal Clinical Hospital No 1 of Chisinau city.

Results: Some medical and social determinants had either a direct or an indirect role in the development of acute pelvic inflammatory disease. In the current study, 40.38% of cases were young women, 59.01% were unmarried, 58.35% of cases – do not use contraceptive measures and 79.18% of patients were from a poor socio-economic background at the time of the study. APID patients had a higher incidence of bacterial vaginosis – 34.93% cases. 52.01% of patients are carriers of chronic diseases and coexistent pathologies are prevailing in one third of the patients included in the study. Approximately 71.89% of patients in the study were hospitalized with a delay of 5 days after the onset of the disease.

Conclusions: We have compiled the contemporary profile for the APID patient – young women, coming from a poor socio-economic background, who do not have health insurance, who are more likely to have a low income, a lower education level, and an earlier start of the sexual life, they tend to have multiple sexual partners as well.

Key words: acute pelvic inflammatory disease, patient profile, medical and social determinants.

Introduction

Acute pelvic inflammatory disease (APID) is the inflammation of the organs of the upper female genital tract. APID is the most common gynecologic pathology observed in females of reproductive age, more than half of them having at least one type of genital infection throughout their life [1]. The number of patients with PID is underestimated because some are not diagnosed due to an asymptomatic evolution, other patients are not hospitalized or may be misdiagnosed and treated at the outpatient care level; these cases are not reported by doctors [6]. According to the scientific literature, the determinants involved in the development of APID can be grouped into: behavioral, social, biological, endogenous, exogenous etc. [8]. A thorough study upon the matter can be justified because inflammatory processes in females with gynecologic pathology tend to become chronic and generalized, with the development of pathophysiological changes and pathomorphologic changes in the affected tissues, with the involvement of the nervous system, endocrine, reproductive, etc in the pathological process [7]. Urogenital infections serve as a starting point for the development of various gynecological and obstetrical diseases [10]. It is considered that after a flare of PID, in 18% of cases, the patients will eventually have an ectopic pregnancy, and 15% of them will suffer from sterility. It seems that the risk of infertility after a single episode of PID is significantly lower for young females (15-24 years) than for women that are older [5]. Women with APID have an increased risk of tubal factor infertility and ectopic pregnancy [3]. The proportion of infertility increases by 15% after one episode of infection; by 30% after two episodes and by 50% after three or more episodes. Women who have had an episode of BIPA

have a 10-fold higher risk of developing tubal factor infertility [9]. The painful pelvic syndrome or the chronic pelvic pain occurs as well in approximately 24-75% of women with PID. The risk of this late-type (delayed) sequelae is 10 times higher in comparison to women who have not suffered from PID[4]. Our study has been focused on analyzing the contemporary profile of the patients who suffer from APID, and identifying the determinants involved in the onset of the disease.

Material and methods

There were analyzed 644 clinical cases of patients that have followed the CDC criteria [2] regarding APID, who were hospitalized at the Department of Gynecology of the Municipal Clinical Hospital No 1 of Chisinau. The aim of the study was to research the contemporary profile of the patient with APID and to identify the medical and social determinants that favor the occurrence of this disease. As criteria for selecting the patients, we have used the CDC standards from USA, where for a diagnosis of APID are being used the following criteria: major criteria - lower abdominal pain, vaginal sensitivity in both annexes of the vaginal tract, pain at the cervix mobilization; Special criteria are ultrasound findings, purulent fluid extraction at culdocentesis, endometriosis identified by endometrial biopsy or laparoscopy; Minor criteria – vaginal discharge, elevated ESR, fever > 38.3°C, leukocytosis, positive laboratory tests for *Chlamydia* or gonorrhea[2].

Results

In order to be able to highlight some correlations between the age of the patients and the occurrence of APID, the patients were divided according to an age criteria. Re-

garding the distribution of the age groups (with an interval of 10 years), we can conclude that the gathered data showed that patients under 25 are the ones that are the most commonly affected by APID, the number of cases being 260 (40.38±1.93%). In the 26-35 years age group, there can be observed a high proportion of patients as well – 184 cases (28.58±1.78%), APID frequency decreases in the 36-45 years age group, comprising 124 cases (19.26±1.56%), and the lowest frequency of APID has been observed in the group of patients after the age of 46 – 76 cases (11.81±1.27%).

The age of starting sexual activity for the patients from the study was as following: up to the age of 16 – 339 patients (52.64±1.97%); 17-18 years – 133 patients (20.65±1.59%); 19 to 20 years – 95 patients (14.76±1.39%); after the age of 21 – 77 patients (11.96±1.28%) ($\chi^2 = 10.8$, $gl = 3$, $p < 0.05$).

As it was demonstrated in our analysis, the highest number of patients had the start of their sexual life before the age of 16 (52.64±1.97%). The number of sexual partners for patients with APID was: 1 sexual partner – 168 cases (26.09±1.73%), two sexual partners – 255 cases (39.60±1.93%), three sexual partners – 91 cases (14.13±1.37%), 4 sexual partners – 53 (8.23±1.08%), 5 partners – 20 cases (3.11±0.68%), no answer to the question – 57 cases (8.85±1.12%) ($\chi^2 = 15.7$, $gl = 5$, $p < 0.01$). We have observed that 65.07% of the patients included in the study, had two or more sexual partners. The types of contraception used by patients are: the male condom – 85 cases (13.19±1.33%), *coitus interruptus* – 171 cases (26.55±1.74%), IUDs – 129 cases (20.03±1.58%), spermicides – 15 cases (2.33±0.59%), COC – 39 cases (6.05±0.94%), no contraception – 205 cases (31.83±1.84%) ($\chi^2 = 18.2$, $gl = 5$, $p < 0.01$).

We have observed that APID has a higher incidence rate in unmarried females (59.01%). A permanent residence has been declared by 357 patients (55.43±1.96%), and a temporary place of residence – 287 patients (44.56±1.96%) ($t = 3.8988$, $p < 0.001$). The studied group, based on their level of education and their job, consists mostly of females who completed the general secondary education – 468 patients (72.67±1.76%), which may account for the degree of self-awareness regarding the possible complications that

may arise after a flare of APID. The study has included 95 students (14.75±1.39%), 89 females engaged in an intellectual type of work (13.82±1.36%), 157 of the females were workers (24.37±1.69%), 53 (8.23±1.08%) of them perform housework, 72 (11.18±1.24%) are unemployed, 5 invalids (0.77±0.35%) and 173 females (26.86±1.75%) were engaged in seasonal jobs abroad ($\chi^2 = 16.2$, $gl = 6$, $p < 0.05$). Unfavorable workplace conditions and occupational hazards may favor the development of APID. 170 of the studied females are working under varying temperatures (26.39±1.74%); 58 of the women are engaged in physical labour (9.01±1.13%); in 26 cases, the work involves contact with toxic substances (4.03±0.77%).

It is known that women from poor socio-economic backgrounds, are more often affected by inflammatory diseases related to unfavorable workplace conditions, occupational hazards, lack of permanent housing, low wages and chronic malnutrition [9]. In order to highlight the socially vulnerable group, we have conducted an analysis of the monthly income for each patient. In this study, a monthly salary of up to 1,000 lei (MDL) has been reported by 230 patients (35.71±1.89%), a salary of 1000-1900 MDL by 37 women (5.74±0.91%); 2000-2900 MDL – 70 women (10.87 ± 1.23%), a salary of 3000-3900 MDL – 19 women (2.95±0.67%), more than 4,000 MDL – 45 women (6.98±1.00%); the average salary being 1094.25±254.63 MDL. From the studied group, 141 women (21.89±1.63%) depend on their partners or other family members; unstable incomes have been reported by 102 women (15.84±1.44%) ($\chi^2 = 15.7$, $gl = 5$, $p < 0.01$).

In summary, we have observed that 79.18% of the total number of women are from poor socio-economic backgrounds, of whom – 52.72% have low incomes, and 21.89% – lack any source of income. 209 of the women included in the study, did not have medical insurance (32.45±1.85%).

The analysis of the medical history, has established the presence of the following medical conditions: cardiac diseases – 115 cases (17.85±1.51%); respiratory diseases – 90 cases (13.97±1.36%); urinary diseases – 183 cases (28.41±1.78%); gastroenterological diseases – 74 cases (11.49±1.26%); dermatological diseases – 57 cases (8.85±1.12%); endocrine disorders – 65 cases (10.09±1.19%);

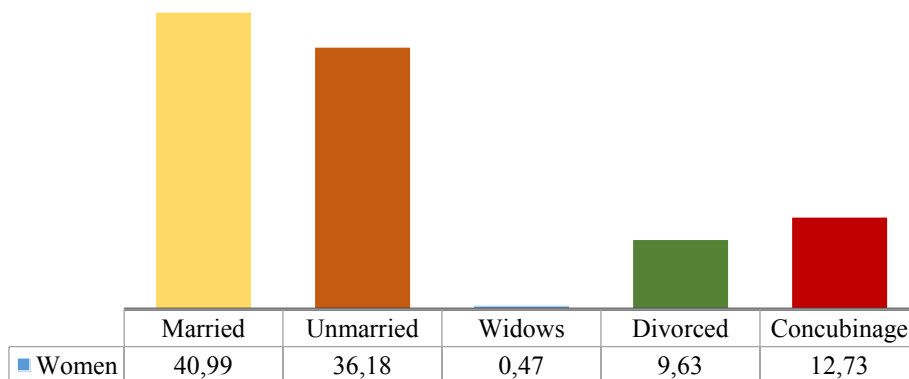


Fig. 1. Marital status of patients with APID (%).

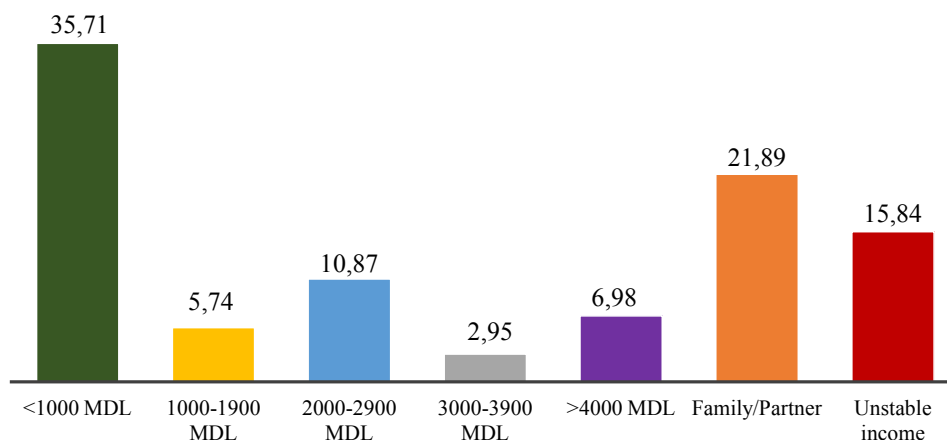


Fig. 2. Monthly income for patients with APID (%).

ophthalmologic diseases – 44 cases (6.83±0.99%); ENT diseases – 23 cases (3.57±0.73%); psychiatric disorders – 8 cases (1.24±0.43%); infectious diseases – 60 (9.31±1.14%); neurological disorders – 32 cases (4.97±0.85%).

We have observed that 243 patients (37.73±1.91%) had two coexistent medical conditions, and in 92 cases (14.28±1.38%) – three or more associated diseases ($t = 9.9327, p < 0.001$). This shows that 52.01% of the patients are carriers of chronic diseases and other coexistent medical conditions, which accounts for a prevalence in a third of the patients. The patients with APID had a higher incidence of bacterial vaginosis, which was observed in 225 cases (34.93±1.88%). Other gynecological diseases encountered in these patients were: genital malformations – 7 cases (1.09±0.41%), endometriosis – 34 cases (5.28±0.88%), uterine myoma – 50 cases (7.76±1.06%), ovarian cysts – 58 cases (9.00±1.13%), menstrual disorders – 88 cases (13.66±1.35%), genital polyps – 19 cases (2.95±0.67%); infertility – 73 cases (11.33±1.25%). Based on the records, we have identified that 381 patients (59.16±1.94%) have given birth, of which 223 women were primiparous (34.62±1.87%) and 158 – multiparous (24.53±1.69%) ($t = 3.9967, p < 0.001$). 263 women from the studied group were nulliparous (40.84±1.91%) ($t = 6.7031, p < 0.001$).

Based on the records regarding the medical history, 208 of the studied patients had abortions based on their own will (32.29±1.84%); 61 women had abortions due to stagnating pregnancies (9.47±1.16%), and 40 women had abortions due to ectopic pregnancies (6,21±0.95%) and for 97 women – abortions were spontaneous (15.03±1.41%) ($\chi^2 = 12.4, gl = 3, p < 0.01$). Of the total number of patients with APID, 40.83% were nulliparous cases, primiparous – 34.62% of patients, 24.53% – multiparous. Abortions have been conducted based on the patients' own will in 32.29% of cases and was the most widely accepted method of family planning.

Due to the analysis of the group of patients using services of systematic dispensarization, we could establish that 144 of them (22.36±1.64%) are being monitored by the family doctor, 45 of them (6.98±1.01%) are monitored by the gynecologist from the system of outpatient care, 74 of the patients (11.49±1.26%) attend annually prophylactic controls at their workplace, and 381 females (59.16±1.94%) do not visit the doctor.

In this study, we have analyzed the type of hospital admission for patients with APID, based on the referrals: for 38 patients, the referral was from the family doctor (5.90±0.93%), for 82 patients – from the gynecologist

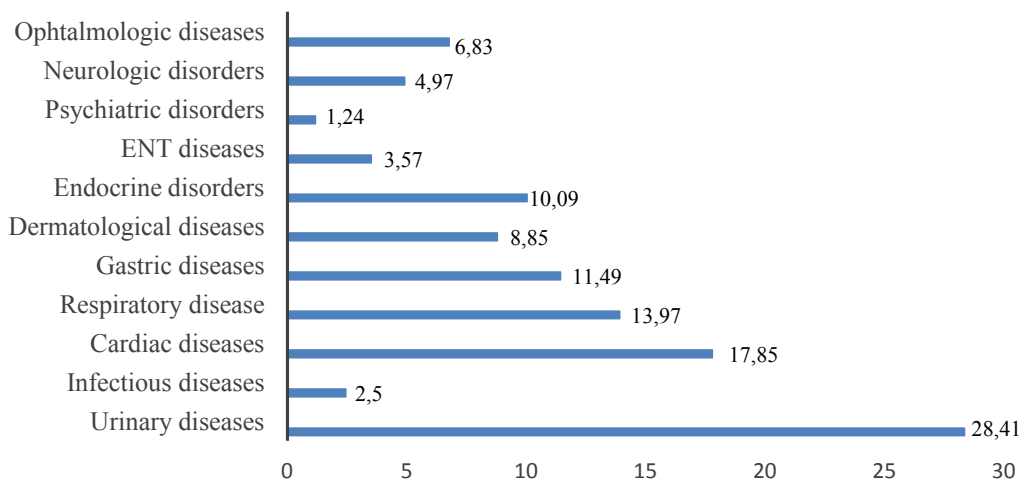


Fig. 3. Medical history of patients with APID (%).

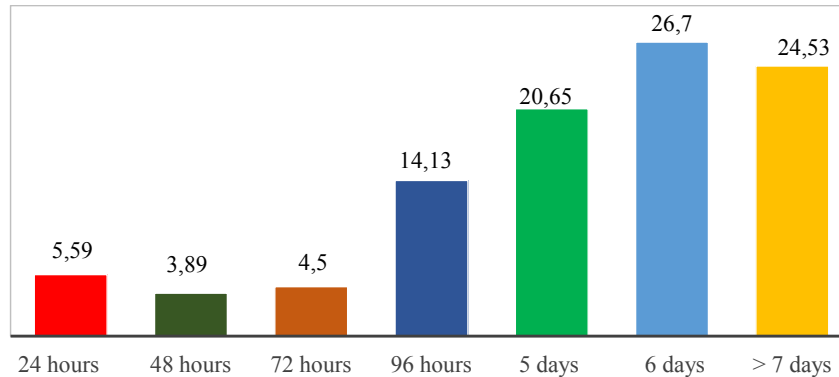


Fig. 4. The time period between the onset of the disease and hospitalization (%).

(12.73±1.31%), for 57 patients – from other medical institutions (10.40±1.20%), and in 70.96% of cases the primary level of healthcare has been avoided entirely. The diagnosis of APID was established by ambulance service teams (47.36%) and by physicians from policlinics (29.03%). The ambulance service was used in 305 cases (47.36±1.97%), self-admission – 152 patients (23.60±1.67%) ($\chi^2 = 16.3$, $gl = 4$ $p < 0.01$).

In addition to this, we have assessed the time period between the onset of the disease and the admission to the hospital, the type of admission and the part of the day when the patient has been admitted. Admission at the hospital, based on the onset of the disease, has been conducted in the first 24 hours – 36 patients (5.59±0.91%), in the first 48 hours – 25 women (3.89±0.76%), during the first 72 hours – 29 females (4.50±0.82%), during the first 96 hours – 91 patients (14.13±1.37%), first 5 days – 133 women (20.65±1.59%), first 6 days – 172 patients (26.70±1.74%), 7 days after the onset – 158 females with APID (24.53±1.69%) ($\chi^2 = 19.1$, $gl = 6$, $p < 0.01$).

The delays observed between the onset of the disease and admission to the hospital may be explained by the patients' misinterpretation of the arising complaints, or it can be associated with atypical forms of APID. Approximately 71.89% of the patients included in the study were given a belated hospitalization 5 days after the onset of the disease, which indicates that women postpone seeking medical help, even when the clinical signs are obvious and the general condition worsens. We also have determined the part of the day, when the patients with APID, are seeking medical help more frequently. The results were as follows: 68 patients have been hospitalized between 00:00-03:00, (10,56±1.21%); between 04:00-07:00 – 95 patients (14,75±1.39%), between 08:00-11:00 – 173 patients (26,86±1.75%); between 12:00-15:00 – 87 patients (13,51±1.35%), between 16:00-19:00 – 56 patients (8,69±1.11%) and between 20:00-23:00 – 165 patients (25,62 ±1.72%) ($\chi^2=12,3$, $gl=5$, $p<0,05$).

Conclusions

The results of the study have allowed us to establish the profile of the APID patient: young women, under

25 (40.38%), unmarried in more than half of the cases (59.01%), 2/3 of patients come from rural areas and live in urban areas at an unfavorable socio-economic level in 3/4 of cases; they lack medical insurance in 32.45% cases; they have an irregular income (25.93%) and do not possess a permanent residence (43.63%). Two thirds of the cases (72.67%) are represented by women with a low level of education, who have admitted starting their sexual life before turning 16 (52.64% cases). One fourth of the patients had more than 3 sexual partners during their lifetime (25.46%).

The data from the study allows us to conclude that APID patients are women of childbearing age – 75.64% (40.34% – nulliparous; 34.63% – primiparous), and in more than one third of the cases they had abortions based on their own will (41.5%). Around 52.01% of these women are carriers of chronic diseases, in one third of the cases there have been identified urinary infection, a higher incidence of bacterial vaginosis and history of abnormal onset of the menstrual cycle.

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New visions upon the neurovascular apparatus of the thoracic aorta

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Abstract

Background: This research has revealed a number of structural features of the innervation and vascularization of human aorta which arouse great interest for clinicians, recently. The following items were highlighted: a) the variability of the intraparietal nervous and vascular elements of the thoracic aorta; b) regional regularities of their histotopography; c) age and individual peculiarities of the glomic structures of the aorta; d) location of the reflexogenic areas of the aorta.

Material and methods: The study was performed on subjects coming to autopsy not more than 24 hours after death. A total 354 human aortas, with mean age from 16 weeks of intrauterine development up to 96 age were examined. A number of research methods have been used: morphometry; histological examination, coloring with Schiff reagent, injective investigation and anatomical dissection.

Results: Dependent and non-dependent gender, age and body type characteristics of the aorta were revealed. Special attention was paid to applied aspects of clinical importance related to the zones of the thoracic aorta.

Conclusions: The variability of the shape, size and location of the glomus structures of ascending aorta was described. Macroscopic, mesoscopic and microscopic structural specific features of the vasculonervous elements of the thoracic aorta were studied from the applied point view.

Key words: aorta, fat body, *vasa vasorum*, glomus, corpus adiposum Rindfleisch.

Introduction

At the beginning of the 21st century, clinicians showed great interest to the cardiac morphology and adipose bodies of the aorta, especially to its ascending portion, which is involved in performing the coronary and cardiopulmonary bypass, as well as the aortotomy of aortic valve plasty (AVP) etc.

Currently, the most common complication of cardiac surgery is postoperative atrial fibrillation [1, 2, 3, 4, 5, 6]. Since 1970s of the previous century, several experimental researches were made [7, 8] to describe the neurogenic tissue within the adipose bodies' content of the heart and of the ascending aorta [9]. J. A. Armour et al. (1997) reported about numerous neurons, which were detected in the epicardic fat pads of human body [10].

Jennifer E. Cummings et al. (2004) reported lower occurrence of postoperative atrial fibrillation (POAF) cases in maintenance of anterior adipose body integrity (and of parasympathetic ganglia within its composition) [7, 10]. J. J. Morrison et al. (2004) conducted and described their own observations regarding the presence of adipose transverse ridge, located on the anterior side of ascendant aorta (AA), whether it is eradicated or impaired during the surgical interventions on the ascending aorta [11, 12]. They assumed that profuse postoperative hemorrhages are caused by some intraoperative actions. The authors detected a number of blood vessels and nerves in the composition of aortic crest, which are relative to the adjacent areas, but did not indicate any glomus structures in adults.

Nowadays, there are several clinical and experimental studies related to POAF, but the results are quite contradictory. A series of questions arise regarding the pathogenesis of aneurysms.

During the 80s of the last century, a series of valuable

works on the biomechanical properties of the aortic wall were published [13, 14], whereas in 2001 this study was extended to fetuses [82] but still there is no data regarding the development of resistant and deformative properties in various aortic diseases.

The last decade of the 20th century and the first one of the 21st century were marked by a huge interest, among the clinicians, regarding the subepicardic adipose accumulation of the ascending aorta [16, 17, 18, 19, 20], but the morphologists did not state any appropriate response with respect to this request. The cardiosurgery guidelines, such as "Cardiovascular surgery" by V. I. Burakovski and L. A. Bokeria [21] and «Safeguards and Pitfalls in operative technique» of Siavosh Khonsary [22] do not report any information about preventing the most frequent and severe postoperative complications in this field.

This very investigation was initiated in conditions when there is an obvious lack of information, which has been obtained through generations of morphologists, referring to morphology of aorta, and does not longer meet the modern demands of cardiovascular surgery and of advanced angiology. The discussed topic is authentic and complements the lack of information from specialized literature and refers to the morphological interpretation underlying some clinical problems. Firstly, the purpose was defined as follows: to determine the possible impact of the vascular nervous apparatus, which may cause postoperative complications and possible aortic disorders.

This paper addresses an urgent current issue in morphology, since the occurrence of cardiovascular pathologies is continuously growing and has become the main cause of death both in developed countries and in developing ones. In Moldova, 66.7% of mortality rate is due to cardiovascular diseases. Difficulties, which may occur in modern diagnosis and curative studies, conservative and

surgical treatment, as well as in the prophylaxis of the aortic disorders are somewhat related to the discrepancy and misinterpretation of the role of different components within the intramural neurovascular apparatus of the aorta in the etiology and pathogenesis of some vascular diseases.

The on-going growth in the number of patients requiring heart surgery, on the one hand, and aortic surgery, on the other hand, as well as the problem of postoperative complications are, unfortunately, quite common, nowadays. Immediate complication is hemorrhage, often abundant, which necessarily require re-sternotomy and the delayed ones – of atrial arrhythmias, which in 7-20% of cases lead to death.

Currently, surgical interventions on various segments of the aorta are widely practiced, therefore an urgent need for diverse data regarding the zone peculiarities of macro- and microscopic structure of the vascular system of the aorta is required, otherwise the desired clinical results will not be achieved. VV condition of the transplantation tissues play a crucial role in a successful treatment (A. H. Cragg and et al, 1983) [23].

The presented study was conducted according to specialists' requirements in the fields of cardiology, vascular surgery (coronary and cardiopulmonary bypass, alloplasty and hetero-plasty of aorta), from the field of mediastinum and spine surgery (spondylectomy) to minimize postoperative complications, primarily in cardiac surgery. The submitted data will contribute to the use of less traumatic treatment approaches.

Material and methods

The study was performed on subjects coming to autopsy not more than 24 hours after death. A total 354 human aortas were examined with mean age from 16-week fetuses up to 96 years. A number of research methods have been used: morphometry; histological examination, coloring with Schiff reagent, injectional investigation and anatomical dissection.

Results and discussion

Through anatomical dissection, we established the connections which occur between vagus nerves and all three cervical ganglia. The sympathetic cervical ganglia branches off ramifications which are connected with vagus and then through vagosympathetic trunk reaches the aortic arch. The left vagus nerve is directed towards the convex and ventral side of the aortic arch, whereas a few small nerve trunks come off towards the anterior aortopulmonary groove and towards the front of the ascending aorta. The right vagus nerve branches are distributed to the back of the arch and ascending aorta.

Hence, the ascending aorta and aortic arch have steady bilateral sources, asymmetrical to the origin of nerve trunks, directed to the heart; each of these trunks is formed by the confluence of the vagus nerve branches

and sympathetic chain. Branches of the intercostal, vagus and splanchnic nerves are oriented towards the descending segment of the thoracic aorta

The existence of aortic nerve connections with all adjacent organs was determined by staining via Schiff's reagent, data which contributes to a clearer understanding of the vaso-vasal and vaso-visceral reflexes, and is useful for medical practice as well.

Nerve elements are not equally distributed in the aortic wall. Thus, the density of nerve trunks per 1 cm² reaches its maximum in the following segments: between the ascending aorta and pulmonary trunk; between the aortic arch and trachea; the posterior wall of the descending aorta.

During our study, the nerve plexus of adventitial aorta was identified, which was well developed had all kinds of nerve elements: nerve trunks, nerve fibers and solitary fibers, free nerve endings, those with well pronounced glial content, Crause corpuscles as well as ganglia, nerve microganglia, solitary and intratrunkul neurons, aortic corpuscles, similar to the carotid ones. Solitary and free sensory nerve endings predominantly occur in the tunica media.

The largest nerve trunks are located in the anterior and posterior aortopulmonary grooves, and on the ventral side of the aortic arch. The spatial arrangement of the nervous structures of the aorta is similar to that existing in other areas of the body, where neurovascular bundles are formed and which commonly contain an arteriole, two venules and a nervous fiber. Haysman phenomenon (dilaceration of nerve bundle) is noted at the intersection of the nerve trunk with a blood vessel.

Another phenomenon is the presence of nerve cells in the above mentioned areas. They are more abundant and disseminated in the outer layer of adventitia, whereas essentially reduced in deeper layers, at the limit of the tunica media. The number and size of intraparietal nerve ganglia on the ventral side of AA are larger than on the dorsal side. The AA convex side contains fewer nerve structures compared to the concave side. In all cases, nerve connections between the nerve trunks of the ascending aorta and the pulmonary trunk are defined.

A specific interest is shown to the location of nerve structures in adventitia, which occur predominantly along the *vasa vasorum* ramifications and vascular networks. Nerve trunks, solitary nerve fibers and receptors are located on their pathway. Nerve plexuses which are macroareolar in the outer layers of the vascular tunica, become microareolar in the deep layers. Micro-ganglia are revealed in places of origin of the aortic arch branches, along aortopulmonary grooves and on the convex side of the ascending aorta.

During its lifetime, aortic intramural nervous apparatus will undergo a series of changes depending on age, or response to pathological conditions. Studying aortic intramural nervous apparatus in various diseases, we found no specific changes. In all cases we defined reactive alterations of the nervous apparatus: hyper-staining of myelina-

ted fibers, varicosities, neuroplasmatic fusions, vacuolation of myelin membrane, brutalization of preterminal and terminal portions of the receptors, mainly of sensitive ones, or their fragmentation. Most people with atherosclerotic aorta, besides the degenerative processes, have also the compensatory terminal devices, characterized by abundant growth or thickening.

Ascending aorta and aortic arch are constant sources of bilateral nerve supply, being asymmetric to the origin of nerve trunks and directed to heart; each of these trunks are formed by the confluence of the vagus nerve branches and those with genesis of the sympathetic chain.

Anterior part of the ascending aorta, is generally innervated by branches of the left vagus nerve, while the posteriorly - by the right vagus nerve. It is necessary to highlight that anteriorly of the aortic arch, connections between these two vagus nerves occur. Nerve trunks of the ascending aorta usually pass anteriorly and posteriorly of AA near the anterior and posterior aortopulmonary grooves. Their branches form an adventitial nerve plexus, whereas ramifications come off into tunica media, where the secondary plexus is formed.

Vago-sympathetic nerve trunks, are formed at the level of concave arch, then directed downwards its origin, along the aortopulmonary grooves, whereas the anterior ones are larger in size. They are followed by numerous nervous micro-ganglia. The trunks are smaller in size on the convex side of AA compared to the concave one. As a result of the morpho-functional peculiarities of the ascending aorta, the nerve trunks and nerve bundles present more or less sinuous pathway.

It is a well-known fact that if the function of the vascular segment is complex, then the nervous apparatus is more varied and intricate. According to our data, the most complex and diverse neurovascular structure was detected in the ascending aorta, particularly in the adipose body of the ascending aorta.

We also observed some regional differences in the placement of nerve elements. Nerve bundles are predominantly directed longitudinally, according to the AA major axis, except for corpus adiposum Rindfleisch' (CAR) where horizontal orientation is present. We identified nerve endings in the CAR structure, characterized by button appearance, in particularly, Krause corpuscles. At the level of adipose body, a larger number of nerve cells and solitary intratrunkal are detected, compared to adjacent areas.

The aortic intraparietal nervous apparatus is well-developed. On segments collected from newborns, we observed an intricate nerve network, which includes nerve ganglia in its composition. Therefore, the aortic wall in infants, including CAR, contains the same nerve elements characteristic of adults.

The peculiarities of AA innervation consists, mainly in the presence of numerous constant nerve structures, of "glomus" type. These are characteristic for both prenatal and postnatal periods of ontogenesis. In other areas, they

are commonly lacking or reduced during lifetime. We stress upon this structural peculiarity, since taken individually, it is characterized by a wide range, regardless of the age group of subjects undergoing study. Hence, this portion of the aorta requires extensive physiological research.

Unlike the glomus structures which are located elsewhere in the aorta, those from the CAR are more numerous and constant. Furthermore, depending on the shape and location of the Rindfleisch' fat body, its corpuscles also vary in location. They may be located on the anterior, on the right and/or posterior surfaces of AA.

According to own observations, these glomus cells undergo reshuffling, although some specifications are required. For example, in adults, the glomus cells are present in CAR, although J. J. Morrison and et al. (2003) stated that glomus structures are missing in adults, being substituted by adipocytes.

The examination of a large number of histological samples, which elucidate the *vasa vasorum internae* (on its pathway), enabled to perform some observations. No glomus structures were found on the incipient portions of VVI pathway, which is 2.0-2.5 cm long, until they penetrate CAR. There are contradictory opinions in the specialized literature, which refer to aortic chemoreceptor apparatus. Thus, the prior purpose of this study was to elucidate the situation, referring to aortic chemoreceptor apparatus.

There were made a series of transverse and longitudinal sections of the CAR. An arterial vessel is present on the cross-sections of the adipose body of AA, passing through the glomus center; on both sides of the arterial vessel, larger venous vessels are often located, compared to those of the artery. In most cases, glomus cells and various-sized supporting cells are contained in the mentioned above structures. Rarely, an arterial vessel of a greater diameter passes through its glomus pole or, in some cases; larger glomus cells may contain two sources of vascularization.

Our results prove a wide variation of glomus structures, located in the adipose body of the ascending aorta. They vary mostly in shape, size, depth, and blood and lymph vessels relationship. There were detected glomus ranging from 100 μ m up to 2 mm - diameter. Usually, smaller glomuses are located deeper, near the aortic media, the larger ones in the outer layers of adventitia, whereas the glomus cells are dispersed within the aortic media. The functional role of these structures presents an increasing interest among practitioners.

A. M. Verity, T. Hughes and J. A. Bevan (1964), when underlining the continuity of glomic-aortic-pulmonary structures, considered it unreasonable to highlight their individual variations. We could not overlook the extremely wide variability of glomus formations enclosed within CAR. There should be noted some changes regarding their structure, in adults. It is not clear why they are found in absolutely healthy and younger people, whereas in pre-senile and senile- aged persons they often show no changes, although persons died of cardiovascular diseases. We can

not confirm that glomus cells disappear along with aging: we have found them in all cases, at various ages. Obviously, the number of sections must correspond to the CAR dimensions. While making the analysis of numerous sections in the more pronounced developing zones of adipose tissue, including the adipose body fat, no cases of failure were recorded. There were recorded dispersed forms of the respective structures, when groups of cells are observed along the blood and lymph vessels pathway.

There was not observed any relationship between the presence or absence of glomus formations and person's gender.

The AA glomus characteristics are similar to the carotid ones: their location is closely allied to arterial, venous and lymphatic vessels; the presence of glomus cells located within the intercellular spaces describes their role as chemoreceptor, it was proved that the location of chemoreceptor structures of the ascending aorta is closely related to the shape, location of the fat body and the advanced development of adipose tissue. Therefore, when the Rindfleisch adipose body is less pronounced anteriorly of the ascending aorta, glomus structures are located in the most developed zone of adipose tissue: on the right or posteriorly of the ascending aorta.

Besides the glomus structures, which have the classic appearance of various-sized carotid corpuscles, there were found agglomerations of various-shaped glomus cells, including dispersed ones. Compact type corpuscles predominate in neonates, whereas in adults a more relevant organizational diversity.

As a rule, those similar to carotid glomus are placed in the outer layers of the adventitia, whilst the other forms within the deep layers. Dispersed cells are detected at the limit of media and adventitia along the lymphatics, in shape of extended cords or rows of glomus cells nests and those which are freely distributed within the intercellular spaces.

The glomus para-aortic structures, which have been well documented and argued by other investigators (Addison and Comroe, 1930 Daly and co., 1970; Coleridge Hazel M. and co., 2016), were not included in the study.

Based on the obtained data, we can state that the number and distribution of corpuscles varies from case to case. However, there is some regularity: aortic corpuscles in the superficial layers are closely allied to the blood vessels, whereas the small glomus structures and their clusters within deep adventitia are placed near the lymphatic vessels. Most commonly, arterial vessels pass through the center of glomus which ramifies. The correlation between the lymphatic and venous vessels present different structural aspect: the glomus cells or corpuscles are paravasally anchored and closely bound to the vessels.

The size of glomus structures of the ascending aorta changes according to age: in the first 3-5 years of life, their sizes grow until the age of adolescence when a slight decrease occurs. In adults there is an increase of linear pa-

rameter values of the glomus structures. The connective tissue lies on the basis of their volume extension. The larger glomus structures are ovoid-shaped, their spatial orientation corresponds to the major axis of the Rindfleisch fat body. Thus, it is perpendicular to the longitudinal axis of the ascending aorta. There can be observed multiple clusters of glomus cells along the lymphatic vessels.

The collected information allows us to complete the classification of glomus cells, which was proposed by A. Hove (1956). It provides four groups of these anatomical structures with the following locations: on the ventral part of right subclavian artery root; on the ventral part of the left subclavian artery root, on the ventral side of the aortic arch, within the connective tissue, located between the aortic arch and ductus arteriosus. Therefore the fifth will include corpuscles incorporated into the Rindfleisch fat body.

The location of the glomus structures closely allied to the blood and the lymphatic vessels, the appearance of dispersed forms, confirm the functional diversity of these structures, mainly the monitoring of the biochemical composition of the blood, lymph and tissue fluids, i.e. of the aquatic medium which surrounds them.

The detailed study of the nervous and vascular apparatus of different portions of the thoracic aorta, showed specific neurovascular complexes only within the fat body structure of the ascending aorta. On minimum amplification their shape resembles "button" nerve endings, whereas on maximum, they become spindle-shaped and contain a nerve bundle surrounded by a vascular network.

Their sizes vary from 0.3 cm to 1.0 cm in length, and between 0.15 - 0.5 cm in width. I met no descriptions of these formations in the specialized literature, which was accessed. In our opinion, such a structural organization is suitable for perception of blood pressure, viz. these are baro- or presoreceptor structures.

In adults, the nerve endings are located in the adventitia and the third external part of tunica media, whereas in fetuses and newborns they often reach the limit of intima. This peculiarity is due to the fact that depressor nerve fibers penetrate the walls of the branchial arches when the medial tunica is still undeveloped. As the vascular wall thickens, the nerve structures move passively from the intima to the external tunica, and, finally, are located in the outer layers of media.

The presence of fat body in the subepicardic layers of ascending aorta formations being similar in structure to that of the endocrine glands may be an argument for performing endocrine functions.

The aortic arch is innervated by branches of the left vagus nerve, except for the posterior part of the right extremity of the aortic arch, which is innervated via right vagus nerve. There were identified sources of innervation of spinal origin, which are directed from the sympathetic chains and intercostal nerves to the arch.

The nerve apparatus of baroreceptor area within the

aortic arch is represented by extended tree-shaped ramifications with free endings, anchored into the deep adventitial layer; by free bush-like nerve endings and lots of compact bushes with glial elements, located in the middle layer of the adventitia. We found no encapsulated endings in the aortic arch. There were detected nerve micro-ganglia in adventitia of aortic arch, accumulations of neurons in the aortic wall; especially in places where ramifications emerge.

The concept approaches on aortic glomus differ greatly. According to data from «Wikipedia, the free encyclopedia, 2015,» there are several small groups of chemoreceptors, baroreceptors and supporting cells along the aortic arch. At the same time, E. V. Trifonov (2015) writes that aortic corpuscles are a single chemoreceptor structure, located in the aortic arch. It is stated that this structure is oval, red-brown, about 5 mm diameter, surrounded by a fibrous capsule. While examining various portions of the aortic arch there was determined the presence of the aortic corpuscles only in the lower wall of the aortic arch, near the arterial ligament in the neonate.

At the origin of aortic arch branches there is a reduced number of nerve endings compared to the adjacent area of the arterial ligament, but they are more crowded than on the anterior and posterior parts of the aortic arch. The maximum concentration of nerve regards to the middle layer of adventitia. The external tunica of the aortic arch reveals numerous nervous micro-ganglia, whereas the characteristics of nervous structures are similar to the area of the anterior aortopulmonary groove.

The descending thoracic aorta is innervated by branches of the vagus nerves, major splanchnic nerves, ganglia of sympathetic chain and intercostal nerves, respectively. The superior cardiac branch top of vagus nerve, depressor nerve, initially it goes isolated, and then it connects with superior cardiac nerve that originates in the superior cervical ganglia.

As regarding intraparietal nerve units of the descending thoracic aorta, they are similar to other portions of this major vessel, except for glomic structures. The latter were not detected.

During lifetime, the constituent elements of intramural nervous apparatus adapt to the conditions of the substrate. In response to the pulsating character of the functioning aorta, the pathway of the nerve bundles appears wavy or spiral. At the level of the ascending aorta, this phenomenon is observed earlier than in the other portions of the aorta.

There were observed some regularities regarding the vascularization of thoracic aorta: along the aortic pathway some characteristics of the vascular bed area were found; *vasa vasorum* density per area unit of the macroareolar network of anterior aortic wall above is more intricate than the posterior one. Vascular loops are elongated, according to the major axis of the aorta.

The obtained results allow us to conclude that two groups of irrigation sources participate in the vascularization of the ascending aorta viz. ascending and descending.

Mainly, Schiff's reagent staining makes it possible to determine a series of new data.

The lower portion of the anterior side is irrigated by branches of the right coronary artery, whilst the posterior by the ramifications of both coronary arteries. The vasculature of AAs anterior part, is carried out by branches of both coronary arteries in 97% of cases, and only in 3% of cases by branches originating only in the right coronary artery. This is determined by the morpho-topographical characteristics of the CAR.

In 45% of cases, at the basis of aortic semilunar valve, there were detected arcuate arteries, which originate in both right and left coronary arteries. There is a high probability that these are present more frequently, or perhaps, permanently. During the study, it was technically difficult to separate, by means of existing methods for determination of these vessels, the base of aortic bulb from the cardiac walls, without damaging the above mentioned arteries.

Their ramifications branch off, predominantly, in basal portions of the aortic valve cusps. In the middle of cusp, the number is reduced whereas they are lacking in the free portions.

The presence of microvascular networks into the aortic cusps proves that the amount of oxygen within valve's tissue exceeds the possibility of its diffusion. Tissue engineering should consider this information while designing the heart valves transplants. Thus, a valve consisting of avascular tissue should not exceed 0.4 mm - thick (approximately). The presence of vascular bed shows that the metabolic activity of the valve is greater than the diffusion might bear it.

The anterior part of AA is irrigated, mostly by the primary branches of the right coronary artery; in other cases, by the auxiliary branches of right auricular artery; and the posterior part- by the auricular branches of both coronary arteries.

There are numerous small branches, which emerge from the bronchial arteries viz. the mediastinal and artery branches associated with the vagus nerve. We detected that in all cases the descending branches go off from the concave part of AA and above the bulb-tubular junction. We did not find any description of them in the consulted bibliography. They present a peculiarity viz. they start from the aortic lumen and do not exceed its wall margins, being located intraparietally, thus represent *vasa vasorum internae*. Their number varies from one to seven, the lumen is larger than that of other sources (in some cases, their diameter reaches 1.5 mm). Normally, they are directed towards the Rindfleisch fat body, whereas larger branches penetrate within it, and branch off depending on the shape and size of this anatomical structure, where it branches to the capillaries, forming anastomoses with all irrigation sources of the AA. This vascular network is richer in the area of pronounced development of fat body, which varies in size and shape from case to case.

It should be mentioned that in fetuses, *vasa vasorum*

interna is the first source of blood supply of AA, which is seen with the naked eye. In 16-weeks fetuses, they form a red stripe, which extends from the concave part of the ascending aorta, and is oriented downwards and to the right, up to the level of connection between the aorta and right atrium auricle. The fat tissue which surrounds these vessels occurs later. In the postnatal period, the most pronounced and larger in diameter AA irrigation sources, are also the mentioned above VVI. In adults, these vessels are often seen under the epicardium, when special techniques are required to detect other types of vessels. It is obvious that these VVI are specifically important to be known, since AA serves as a gateway to heart surgery. In order to facilitate the access, body fat is removed before these procedures are applied.

The blood pressure within *vasa vasorum interna* with the AA genesis is high. Their impairment may result in profuse postoperative bleeding (in cardiac surgery), which requires re-sternotomy. The recorded cases answer somewhat the questions of the clinicians in recent years, thus showing the importance of body fat study before choosing a place for access. Therefore, special efforts are required to maintain the integrity of CAR.

Based on the information we have about *vasa vasorum interna* (VVI), we may claim the following: the blood pressure within these VVI is higher than in the other sources of AA vasculature. This fact somewhat reduces the effect of intraluminal pressure on the AA walls. Thus, namely these sources are essential for irrigation of reflexogenic area. The premature development of VVI, unlike other descending irrigation sources of this aortic portion (bronchial and mediastinal arteries, artery associated with right vagus nerve) confirms this idea.

The appearance of CAR vascular bed depends on the shape and movement of this fat structure. The VVI pathway of AA differs in different locations and shapes of adipose body. In cases if CAR gets into contact with anterior adipose body above (from the anterior aortopulmonary groove), usually these vessels are not seen with the naked eye. They are rooted initially in the fat tissue of the latter, and then occur under the epicardium and above the AA adipose body.

They can be fully revealed only by means of special investigation techniques. In cases when CAR has different configurations, except for the adipose pad, it starts anteriorly of the ascending aorta and does not contact with CAA, *vasa vasorum interna* is directed downwards, a bit to the right from its origin, on the concave side of the ascending aorta and parallel to the anterior aortopulmonary groove. They neither do nor branch off until they enter into CAR, and are major-type inside. They branch off, thus forming anastomoses with other AA irrigation sources.

In cases of fat pads, the VVI pathway is quite different. It is worth reminding, that this form of CAR is found only on the right and posterior parts of the ascending aorta. We have not found any case of its location anteriorly of hu-

man ascending aorta. *Vasa vasorum* is oriented obliquely (down to the right) from its origin, forming a reclining angle against the major axis of AA the angle is sharp when CAR is placed on the right side and obtuse if it locates posteriorly.

In such situations, VVI does not branch off until it enters into adipose body, but once inside it breaks down into fan-shaped branches. These branches form anastomoses among themselves and with other sources of irrigation, forming a dense vascular network, similar to a ball. These peculiarities of vascularization among the adipose pads should be considered when determining the place of surgical incision in the ascending aorta.

The statistical analysis of the obtained information has indisputably demonstrated that the oblique pathway of the VVI is typical for the CAR localizations on the right and left sides of AA, when the fat body is placed differently; whereas when the anterior surface of the ascending aorta is involved, we can observe that VVI pass from their origin downward along the AA concave surface till the fat body, and then they are enclosed within this structure.

Currently, the underestimation or ignoring the existence of VVI during the cardiac surgeries leads to many casualties in the postoperative period. Recently, due to the lack of information about these blood vessels, discussion arose about, "enigmas" which occur in zone of ascending aorta (Jokz C. Lindsay H., 2004; J. J. Morrison, Codispoti M., Campanella C., 2003, 2004) [11, 12, 24].

We noticed that the presence of multiple VVI is typical in cases where AA is sharply curved. Under these conditions, one or two larger vascular trunks are directed to the fat body in typical manner for each type of it. Other branches move to the convex part of this aortal segment, where the radial compression is at highest. No glomus structures were detected throughout these branches.

The prominent density of *vasa vasorum* in AA and the presence of blood supply sources, originating directly into the aortic lumen (*vasa vasorum interna*) is a necessity to ensure adequate nourishment of the aortic area with the largest diameter, wall thickness and the highest radial compression. We conclude that, functionally, VVI which is embedded into fat body differs from those that supply the convex part of the ascending aorta. The first provide continuous irrigation of the chemoreceptor structures, whereas others – continuous irrigation of the aortic area, which undergoes the largest compressive forces and warrants its strength throughout lifetime. The findings regarding the pathway of *vasa vasorum interna* in different types of adipose bodies, prove the importance of their intraoperative visualization before making the incision of aorta, in order to prevent bleeding that turns to be life threatening.

It should be noted that the densest vascular network that contains the greatest number of anastomoses in human aortic wall, is localized at the level of the fat body in the ascending aorta. The list of irrigation sources of ascending aorta, according to the decrease of the lumen di-

ameter up to Rindfleisch body fat is as follows: *vasa vasorum interna*, right and left coronary arteries, the artery associated with right vagus nerve, bronchial arteries and mediastinal arteries. How can we explain the existence of numerous irrigation sources of ascending aorta?

The results of experimental research of several scientists showed that radial compression force of the AA wall is four times larger than in the abdominal aorta. Such extreme operating conditions of this aortic portion require particular vascularization. The presence of VVI provides blood flow both during heart systole (via descending sources) and diastole (via descending – branches of the coronary arteries).

Previously I have described that the formation of this network is performed by branches of lots of sources, including *vasa vasorum interna*. The latter provides the necessary blood pressure level to properly irrigate the reflexogenic area, despite the high luminal pressure in this portion of the aorta. The data available on the VVI of AA makes it clear the necessity to modify and improve cardiac surgery techniques.

As about the causes of profuse postoperative bleeding, after the patient has undergone surgical interventions on AA: irrigation sources form anastomoses not only between them, but with branches of intercostal and phrenic arteries (branches of descending aorta), as well as, at the transitional place of the parietal pericardium into the visceral.

We can not doubt the experimentally obtained results, such as heart rate and blood pressure alterations as a result of excitation of the aortic arch with venous blood [25]. This experiment also demonstrates that there is a zone or hemo-receptor zones within the aortic arch, but we can not actually prove their specific localization. The fact that we found no glomus structures in the descending thoracic aorta and arch level does not confirm their absence, in adults. The aorta is a large sized vessel and requires the study of any micro unit per mm² of the thoracic wall in order to detect microscopic configurations incorporated into the walls of the vessel, the fact which, unfortunately, was not possible to perform during the current study.

We have also complemented the theoretical and practical importance of VVI information, confronting our obtained data (*vasa vasorum interna* in ascending aorta area) with Comroe's reports. We noticed that their origin corresponds to the indicated level and they have positive response to the introduction of Lobeline cannula within experimental investigations conducted by this remarkable scientist. The localizations of numerous structures similar to carotid corpuscle along the VVI of AAs is a valuable argument to conclude that reflexogenic chemoreceptor area of the aorta is localized in Rindfleisch fat body.

The branches of the intercostal and bronchial arteries to the aortic arch are various in number, and branching manner. There is also a third group of branches - from the brachiocephalic trunk. The latter branch off in adventitia of aortic sac derivatives: on convex surface of the central

portion of the aortic arch and the incipient portion of arch, then extend anteriorly of the distal AA portion. The pathway of TBC branches changes in here, some of them turn to the convex side, the others - to the concave ascending aorta, and form mutual anastomoses posteriorly of AA. They do not form anastomoses with vascular sources of proximal or distal portions of the AA. Often, the branches of the bronchial arteries assist in the formation of anastomoses on the posterior part of the arch. Most commonly, several branches go upwards starting from the third left intercostal artery and move towards the anterior side of the distal portion of the arch. The branches of left bronchial artery supply blood to the concave side of the arch and lower portion of its posterior side.

Summarizing, we can state the following: the convex portion of the aortic arch is irrigated by an artery originating from the base of brachiocephalic trunk, whereas the concave (initial and terminal portions of the arch) – from the bronchial arteries, which vary greatly in number in the left third intercostal artery. Bronchial arteries form anastomoses with the branches of the brachiocephalic trunk both on the anterior and posterior parts of the aortic arch.

In some cases, there were found branches at the base of the left common carotid artery, which start from the lumen of this artery, pass through its adventitia and branch off in the adjacent area of the aortic arch. There was detected a clinically significant peculiarity of the vascular bed and the intramural aortic arch. The irrigation sources of aortic arch extend about 1 cm proximally to the origin of the brachiocephalic trunk and distally of the isthmus.

The morphological characteristics of the areas of the aortic vascularization are determined both by its ontogenesis (the presence of multiple sources of development) and various hemodynamic conditions, which occur along them. Thus, the bulbar portion of ascending aorta, which is a derivative of arterial trunk is vascularized by coronary arteries; its tubular portion, mostly the aortic arch, derivatives of aortic sac are vascularized by branches of brachiocephalic trunk and *vasa vasorum interna*; the concave side of the arch, which develops from the IVth aortic arch is nourished through the aortic bronchial and mediastinal branches; the distal portion of the arch, as well as the descending thoracic aorta, derivatives of dorsal aorta, are vascularized from intercostal arteries. In all cases, there is a poorer irrigation of the adjacent areas of the arch, both from the ascending aorta and descending thoracic aorta. Anastomoses rarely occur between sources of the arch with those of AA and the descending portion. This fact has been noticed both in adults and in children.

While comparing this situation with clinicians's data in the field of cardiovascular surgery, regarding the occurrence of dissecting aneurysms, we may conclude that this is not an accidental coincidence. Namely, these aortic walls are poorly vascularized, thus more commonly affected by the aneurysms. Hopefully, the stem cells usage created by

modern technologies will enable the strengthening of the aortic wall with *vasa vasorum*.

There was detected, within the aortic arch, an autonomous network of vascularization from *vasa vasorum internae* system in the adjacent zone of the aortal ending of arterial ligament. The baroreceptor area is irrigated by 2-3 vessels which originate closely the target area. We have not recorded any anastomoses of these vessels with other irrigation sources of aortic arch.

At the level of the aortic arch, particularly in its convex portion, the blood vessels form multiple loops and rings oriented predominantly transversely. On the convex side, the loops are macroareolar and on the concave – of smaller size and more round-shaped.

VV of the descending thoracic aorta originate from the posterior intercostal arteries. Being placed posteriorly to the aorta, they form a denser network on the dorsal side of aorta. Thus, the descending thoracic aorta is vascularized by the branches of the major intercostal arteries of 150-200 mcm in diameter. Frequently, their segmental character was observed on the ventral side of the descending aorta, which was not found in the arteries on the posterior part of the descending portion of the thoracic aorta. The segmental contralateral anastomoses are well pronounced on the anterior side of the descending thoracic aorta, which are missing or have a very small diameter on the posterior area of the same aortic portion.

It was noticed that arterial *vasa vasorum* are easily distinguished from veins by a relatively rectilinear pathway, whilst in the veins it is more sinuous type. Furthermore, the arterial vessels are less numerous, with a smaller lumen compared to veins.

The data regarding the depth of *vasa vasorum aortae* localization are as follows: the adventitia is completely vascularized, the tunica media – only 2/3 externally, intima is normally not vascularized. The depth of the *vasa vasorum* localization is reduced as the distance from the cord decreases.

As referring to arterial bed of the descending thoracic aorta, it should be mentioned about the postnatal changes of primary *vasa vasorum*, which have a diameter of 150-200 mcm in newborns and are segmental ones; in adults they are relatively narrow, fewer in number because of the partial loss of their segmental character. In the latter cases, bilateral longitudinal arterial trunks are detected, which are anastomosed through cross arteries and form contralateral anastomoses.

The distribution of *vasa vasorum* of the descending thoracic aorta is not uniform. The aortic vascular network is richer on the posterior side, while the larger vessels occur mostly on the anterior side. *Vasa vasorum* form two similar networks to those in AA and arch, whereas the veins drain into the intercostal ones.

Numerous observations during the study reveal the presence of special sources of blood irrigation of the glomus structures of the ascending aorta, with specific histotopog-

raphy. The glomus cells of adipose body of the ascending aorta are vascularized from *vasa vasorum internae*, which start on concave side of AA and above the bulbotubular junction and then go through their center, having numerous branches that enter the glomus. The stained samples via Schiff's reagent, display multiple arterial sources which approach glomus from different directions.

The structure of the vascular bed of the baroreceptors zone of aortic arch, placed near the site of insertion of the arterial ligament, differs from the fat body of the ascending aorta. A dense vascular network is detected in this area, consisting of vessel branches of specifically *vasa vasorum internae* category that starts from the adjacent portion of the concave part of aortic arch. No anastomoses with other sources have been revealed. Hence, both reflexogenic areas of the aorta are irrigated by *vasa vasorum internae*.

It is a well known fact that the structure of the aortic walls is constantly reshuffling. The aging changes of substrate, definitely lead to modifications in the vascular bed [14].

It is to be noted, that the number of capillaries in the tunica media of aorta decreases several times since the first age of maturity compared to the first age of infancy. Along the aging process, the number of blood vessels per area unit decreases, whereas their diameter essentially increases. As a result of these changes, the quality of aortic wall vascularization decreases, a fact which may explain the restructuring of vascular wall and its self-destructive processes due to aging.

In elderly, the arterial blood vessels are convoluted, larger in size and occur in deeper layers of adventitia. The capillaries show a sinuous pathway, as well. Depending on the age, *vasa vasorum* density gradually decreases (six or more times). The vessels become deformed, and are missing in the region of massive mineral deposits, whereas occur in a higher number in the areas of early calcium deposits.

Hence, the blood vessels can be detected only in the external tunica of the aorta in the first three years of postnatal development. Around the age of four, they penetrate the tunica media, then at 10-11 years old they reach its middle layers and finally in adolescence (16-17 years old) into the deep layers.

In the first period of maturity (22-35 years), a decrease in number of capillaries in tunica media of the aorta was detected; the change of this indicator is essential, for example, in comparison with the first period of childhood. Hence along with aging, the number of blood vessels per unit area decreases in the wall of thoracic aorta, while their diameter increases essentially.

The diameter of vascular sources and density of vascular plexus of the aorta are reduced proximodistally. In cross section, there is a dense vascular network seen on the background of multilayered aortic vascular plexus, which is located between the adventitia and aorta media.

Because of this aging reshuffle, the quality of human

aortic vascular wall is lowered and the incidence of various pathologies increases. Thus, the formation and differentiation of the structural units of intramural vascular bed of the thoracic aorta continues in postnatal ontogenesis. Simultaneously, certain functional mechanisms are established which adapt to hemodynamic conditions and are constantly changing during lifetime.

It was identified that each portion of the aorta is characterized by specific histotopographical features of circulatory bed elements; by vascular network density and the presence or absence of *vasa vasorum interna*. Generally, there is a clear regularity: the total thickness of the wall of the aorta determines the penetration depth of the blood vessels into it.

The aortic function is not limited only to the distribution of blood to various organs. The aorta is an essential component in the biomechanics of the blood circulatory system. Thus, its resistant-deformative qualities, actually, deserve particular attention. Investigations of physical and mechanical characteristics of blood vessels (tearing strength, resistance limit, maximum relative extension, Young's modulus) have both theoretical and clinically applicative value. For example, the data about the resistance of blood vessels may arise interest to planning of high-speed flight. The sudden changes in biomechanical properties of vessels may occur during pathological processes: atherosclerosis, hypertension and others. Therefore, information about the condition of the vessel wall is necessary, for example, in checking up new drugs, in reconstructive surgery of vessels, including their prosthesis (viz. prosthesis manufacturing).

We started to examine this aspect, while facing a clinical unexplained problem: the incidences of sudden rupture of the ascending thoracic aorta without a history of aneurysms, injuries, infections, dissections or any previous surgery; it is a very rare event, but potentially fatal.

We studied the AA portion, which corresponds to the area of Rindfleisch fat body in atherosclerotic coronary damage. The segment which was selected, presented clinical

value and the vascular sources were easily visible and documented. But, since Rindfleisch fat body and aortocardiac fat bodies were not given proper attention, so far, we used the tensometric study AA fat body zone. Since, we focused mainly on *vasa vasorum* of AA, especially in the CAR zone, we admit that they somewhat depend on the tensometry parameter values of the target body area.

In order to determine a certain link between the normal and abnormal states of *vasa vasorum* in this area, and in order to compare the two states, we established the same tensometry values in people who underwent ischemic cardiosclerosis, after which *vasa vasorum* was affected. We determined some differences in the given conditions (intact and affected *vasa vasorum*), then included this information into the study, which confirms the practical value of the present research.

The information obtained by means of determining the resistant-deformation characteristics of the aortic wall in the group of people with affected coronary vessels and in the control group, demonstrates, that both the resistance limit and maximum relative extension of the samples taken from the Rindfleisch fat body in people with heart disorders, caused by atherosclerosis of the coronary arteries, presented lower values, compared to the control group. These individuals had a lower stiffness coefficient – 1.095 gf/mm² compared to 1,271 gf/mm². Obviously, one of the causes of aortic aneurysms, besides the hereditary diseases, characterized by connective tissue disorders (Marfan syndrome, Ehlers-Danlos vascular syndrome, Loyes-Dietz syndrome) may be the real state of *vasa vasorum*.

The results of the statistical analysis of biomechanical properties of AA have proved the following: during the process of impairment of aortic *vasa vasorum*, a decrease of biomechanical basic indexes occurs (tearing strength, resistance limit, maximum extension and stiffness coefficient), both in men and women (p <0.001). Statistically, the changes of resistance-deformation characteristics of the aortic wall occur during the atherosclerotic impairment of vascular sources, mainly of the coronary arteries.

Table 1

The basic resistance –deformation properties of the ascending aortic wall in the CAR zone, independent of gender

Indices	Group	N	Index value	Dev/ std.	m	p
Mean age (years)	control	10	54.5	12.232	3.868	>0,05
	experimental	10	58.8	10.963	3.467	
Tearing strength, kg/mm	control	10	1.8767	0.10059	0.03181	<0,001
	experimental	10	1.5146	0.06865	0.02171	
Resistance limit, kg/mm ²	control	10	0.092	0.00281	0.00089	<0,001
	experimental	10	0.075	0.00182	0.00058	
Maximum relative extension, %	control	10	84.02	1.265	0.4	<0,001
	experimental	10	59.03	1.812	0.573	
Stiffness coefficient (gf/ mm ²)	control	10	1.0955	0.03263	0.01032	<0,001
	experimental	10	1.2721	0.05329	0.01685	

Although this fact is not significant, it has several explanations: rich anastomosis of the coronary vessels to other sources of irrigation; presence of VVI with larger lumen and higher blood pressure.

Conclusions

1. The nervous apparatus of thoracic aorta forms connections with all adjacent organs.
2. The intramural nervous apparatus of the aorta includes trunks and nerve fibers, nerve ganglia and microganglia, clusters of nerve cells, solitary nerve cells, glomus structures and nerve endings: encapsulated (presented by the Krause corpuscles) and unencapsulated.
3. The density and variability of nerve and vascular elements are the most obvious in the fat body composition of AA. Only in its composition there were detected specific neurovascular complexes, formations and structures specific for endocrine glands and glomus structures different in shape, size and location.
4. The proximodistal gradient of innervation and aortic vasculature is obvious.
5. Sources of vascularization of various portions of AA, arch and descending aorta, being ontogenetic derivatives of different structures, do not practically form mutual anastomoses in postnatal period.
6. Late onset of atrial fibrillation after a surgical intervention can not be justified by impairment of nerve and vascular structures embedded in it, whereas the profuse bleeding results from *vasa vasorum internae* injury with high blood pressure and rich network of anastomoses.
7. Impairment of certain atherosclerotic vascular sources leads to the decrease of main tensometric values of the aortic wall.
8. VVI is a mandatory source of vascularization of reflexogenic zones, whilst histotopographic organization of the vascular bed within the chemoreceptor and baroreceptor zones is different.
9. The comparison of the unprecedented obtained results with the experimental data of Iu. Comroe indicates the presence of reflexogenic zone at the level of AA fat body [27].

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Impact of primary health care sector in the detection of tuberculosis on the model of Chisinau city

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Abstract

Background: Tuberculosis detection represents the major challenge in actual health care system, recognised in the Republic of Moldova and worldwide.

Material and methods: 101 pulmonary TB patients diagnosed in the period 1.1.2015-31.12.2015 in Chisinau were enrolled and distributed into two groups: the 1st group consisted of 73 patients with pulmonary TB detected in the frame of primary health care sector by general practitioners as symptomatic patients, and the 2nd group consisted of 28 patients with pulmonary TB detected by general practitioners by active way of screening.

Results: Patients from both groups were enrolled in a similar proportion according to the demographic characteristics (urban/rural residency, civil and educational status). Two thirds of both groups were uninsured, but the economic state was lower in the group of patients detected as symptomatic cases. Chronic alcoholism, drug use, history of imprisonment, migration, comorbidities were established in a similar number of cases from both groups. Despite the fact that high risk groups (TB contacts and HIV infected individuals) must be screened actively, their rate is statistically higher in the group detected by passive way. Smear positive results, extensive pulmonary infiltrates and lung parenchyma destructions predominated in the group detected by passive way. The rate of patients that successfully finished the treatment was similar in both groups, but deaths were registered only in the group detected by passive way.

Conclusions: The Republic of Moldova registers a continuous decrease of epidemiological TB indices due to the reduction of high risk groups designed to be investigated and the insufficient use of active screening.

Key words: tuberculosis, primary health care sector, risk groups.

Introduction

Tuberculosis (TB) represents a major threat to public health worldwide. The internationally approved strategy in control of TB, defined as End TB Strategy combines health promotion, disease prevention, case detection and patient management at the first encounter [2, 9, 12]. In the countries of Eastern Europe, Baltic States and the Commonwealth of Independent States (CIS) the diagnosis and treatment are performed in the specialized TB services. TB control system from those countries follows a three-tiered structure, incorporated at the national, regional and district level. The system of national TB control services consists of a network of TB facilities and health care institutions. However, for the successful disease control, entire health care sector must participate in TB control practices [11]. Early detection and referral for out-patients treatment represent the frontline where health care providers are involved, in a way reducing the burden of TB at the community level. Primary health care providers (PHCP) are health workers: physicians-general practitioners (GPs), nurses, auxiliaries and community workers who serve frontline providers, responding to the health needs of the community [7]. PHCP are the first to meet a TB patient, before the diagnosis is established [6]. Those providers represent the level where early detection must be performed and the efficacy of TB control activities has bigger impact (considering that a person with an undiagnosed active TB will infect on average 10-15 other people per year). Specific roles of PHCP differ according to regional or national guidelines,

but they must accomplish the following functions established by the WHO Guide on TB Control for Primary Health Care Providers for countries localized in the WHO European Region with high and intermediate TB burden: 1. Suspect the disease in patients with symptoms suggestive of TB; 2. Ensure collection of sputum for microbiological examination for acid-fast bacilli from the patients with productive cough lasting more than 3 weeks; 3. Send the collected sputum to diagnostic laboratory for identifying acid-fast bacilli; 4. Order to perform chest X-ray examination of suspected patients; 5. Refer suspect individual to the specialized services for diagnosis and treatment; 6. Communicate to patients that the disease is curable and emphasize the importance of a regular and complete treatment; 7. Communicate with specialized organizations about the patients referred for diagnosis and treatment; 8. Perform screening of close TB contacts; 9. Educate general community about the signs and symptoms of TB; 10. Provide directly observed therapy till completion during the continuation phase; 11. Report complications, drug side effects, default of specific treatment to TB services; 12. Complete administrative forms and send them to the TB services; 13. Monitor high risk groups according to the national recommendations; 13. Perform BCG vaccination and tuberculin skin testation of children-closed contacts with TB patient [7]. Some specific recommendations for Moldovan PHCP were included in the national guide for "Tuberculosis Control at the Primary Health Care Level : 1. Ensure the chemopreventive treatment of contacts from 0-18 years old and HIV infected contacts; 2. Identify indi-

viduals at high TB risk according to the national guideline to perform examination (chest X-ray examination in adults and tuberculin skin testation in children); 3. Perform collaborative activities with local NGOs in disease prevention; 4. Support TB patients in association with the community, public local authorities and governmental institutions [6]. The Moldovan guide emphasizes some regulations of active screening to be performed by GPs from PHC sector: 1. Perform clinical examination of high risk groups of adults and children every 6 months; 2. Order a chest X-ray and microscopic examination of the sputum of individuals with suggestive signs and symptoms; 3. Order a chest X-ray in adults and tuberculin skin test in children from high risk groups at least one time in 12 months, even if there are no clinical signs suggestive for TB; 3. Perform the clinical examination annually to individuals included in the dangerous groups; 4. Perform a chest X-ray in patients from dangerous groups with suggestive signs and symptoms. 5. Perform a chest X-ray in individuals from contingency groups before the enrollment into the work field and one time per year. There are specific regulations that must be performed by PHCP in the frame of passive screening: 1. To identify and send the patients with suggestive signs and symptoms for microbiological examination for acid-fast-bacilli. 2. To send the patients to the pneumophthysiologist after performing the investigations and the non-specific treatment [6].

In the majority of countries of the Commonwealth of Independent States the vertically structured specialized TB care services are unsatisfactorily coordinated with the PHC system; however the activities of both sectors must be interdependent for a better TB control. In this context, it is important to underline that rapid progress in control of TB occurs only in health systems where TB activities are integrated in PHC, and TB case detection and management procedures are implemented in the frame of the entire population [8]. The major role of PHC providers in the Commonwealth of Independent States is to detect TB suspects and referring them for diagnosis and treatment to specialized organizations. The actual challenge represents the extending ambulatory TB treatment, and replacing the in-put (hospital based case) with out-put (ambulatory care), duty that will become even more important than the detection of new patients with active disease [5].

Primary health care providers (PHC) are important actors in TB control in the Republic of Moldova, because the majority of TB patients report their first visit to GPs due to suggestive clinical signs and symptoms [6]. As a rule, PHC is the first level where individuals, family and general community receive health care, being the most accessible and affordable care for the majority of people of our community. PHC performs the following activities: health education, promotion of proper nutrition, maternal and child care, immunization against major infectious diseases, prevention and control of endemic diseases, treatment of common diseases and provisions of essential drugs. For an

optimal ongoing of activities, PHC must be sustained by the referral systems – hospitals and ambulatory specialized organizations, as well as by the civil society organizations [8]. One of the most important activities which must be performed by the PHCP is the good communication with patient and entire TB specialized service. It starts at the community level and represents the communication with patients with symptoms suggestive for active disease. PHCP will order to execute sputum smear investigations, molecular genetic assay – GeneXpert MTB/Rif (semi-quantitative, nested real-time polymerase chain reaction for detection of *Mycobacterium* DNA and rifampicin resistance mutations of the *rpoB* gene) and chest X-ray to all symptomatic patients. At this level, the consultation of TB specialist will be also required and the symptomatic patients will be referred to the TB services for further examinations. Within 2-3 days, if the investigations confirm active disease the pneumophthysiologist will start the specific treatment, diminishing the risk of TB transmission in the community [6].

The health system of the Republic of Moldova is organized around the principle of universal access to basic health services and equity in health services [3]. It is financed by the state and by citizens, through mandatory health insurance mechanism. The Ministry of Health is overseeing the health system, demonstrating the responsibility for the management of health services [10]. However, the financing of most health services lays on the National Health Insurance Company. In the Republic of Moldova, PHC is provided by family medicine centers, primary care centers and health centers. Actually, our country registers significant success in reorienting the health system towards primary care facilities. In rural localities primary care services are provided by the family doctor offices and health centers, but in urban areas they are performed by the big family centers – Territorial Medical Associations (TMA), former “policlinics”. The primary health care level consists of 37 family medicine centers in the Republic of Moldova, including 216 health centers, 556 family doctors offices and 359 health offices. There are also 46 autonomous health centres, covering 71 family doctors offices and 44 health offices. In Chisinau, there are 5 TMA, covering 12 family medicine centers, five consultative and diagnostic centers. Family medicine centers and consultative centers provide family medicine and specialized outpatients services. A health centre service has at least 4500 inhabitants and three family doctors. One family doctor services a population of 900-3000 inhabitants, but the official norm of patients per a family doctor is 1500 patients [3]. PHC demonstrate an important role in providing preventive and health promotion services. The WHO study “Evaluation of the structure and provision of PHC in the RM” determined that the number of patients referred to medical specialists in our country is very high [10]. Considering this fact, the resources of PHC are not efficiently used, avoiding them through ambulance services that increase the risk of hospi-

talization. There are multiple causes for avoiding the PHC facilities in the RM: long waiting times, limited and unsatisfactory quality of provided services, in this way contributing to a higher addressing of the population the specialists and hospital services. The current management of the patient starts with the first consult of GP, who decides if a further referral is needed to a specialized ambulatory clinic or a district hospital. The next step represents the decision of the specialist to investigate, or to admit the patient into the specialized hospital or to refer the patient back to the family doctor, who is responsible for monitoring the accomplishment of the recommendations. In urban areas, people call for emergency health care – ambulances, being directly transported to hospitals. There are patients, for example with active TB, who are directly self-referring to tertiary – hospital facilities [10]. Considering exposed data it can be concluded the major role belongs to PHCP in TB control activities at the community level.

So, the aim of the study was the assessment of the major epidemiological indices dynamics in the period 2013-2015 and the impact of primary health care sector on the detection of pulmonary tuberculosis cases and treatment outcome in 2015, in Chisinau. Established objectives were: 1. Assessment of dynamics of epidemiological indices in Chisinau, during 2011-2015. 2. Assessment of general, socio-economical and epidemiological risk factors of pulmonary TB patients detected by GPs according to the way of detection; 3. Evaluation of case-management, clinical aspects, radiological aspects and treatment outcome of pulmonary TB detected by GPs according to the way (passive and active) of detection.

Material and methods

It was performed a retrospective selective, descriptive study targeting social, demographic and economic peculiarities of 101 patients with pulmonary tuberculosis diagnosed in Chisinau city in the period of 01.01.2015-31.12.2015. The informational system for monitoring and evaluation of tuberculosis cases (SIME TB) was used for the patients' selection. Data were extracted from the statistic templates within the frame of tuberculosis case registration – F089/1-e “Declaration about patient's established diagnosis of new case/relapse of active tuberculosis and restart of the treatment and its outcomes” and F090 “Declaration and evidence template of tuberculosis cases”. Included criteria were: age > 15 years old, new case of pulmonary tuberculosis (patient never treated for TB, or has taken anti-TB drugs less than one month.), established through positive GeneXpert MTB/RIF assay, and signed informed consent. The investigational schedule included demographic, social and epidemiological data: sex (male/female ratio), age (distribution in age groups according to the WHO recommendations), (urban/rural residence, country of patient's origin), educational level, socio-economic status (employed, unemployed, retired, disabled,

student), health insurance status (uninsured, insured), migrational and detention history, presence of high risk (close contact, comorbidities: HIV-infection, psychiatric diseases, immune suppressive treatment), type of infectious cluster, health care seeking behavior, way of the patient's detection. All selected patients were diagnosed and managed according to the National Clinical Protocol 123 “Tuberculosis in adults”. Enrolled patients were distributed in two groups: the 1st group constituted 73 patients with pulmonary TB detected in the frame of primary health care sector by general practitioners as symptomatic patients and the 2nd group constituted 28 patients with pulmonary TB detected by general practitioners by active way of screening. Statistic assessment was carried out using the quantitative and qualitative research methods. Statistical survey was performed using Microsoft Excel XP soft.

Results and discussion

According to the published data by the Moldovan National Centre for Management in Health during the period 2011-2015 it was registered an important decline (with 23/100.000) of the new case incidence in Chisinau city from 76/100.000 in 2011 to 53,9/100.000 population in 2015. The total number of new pulmonary TB cases decreased from 602 in 2011 to 435 in 2015 [1]. By PHCP were detected 213 (48,96%) new pulmonary TB cases in 2015. In the frame of passive way of screening GPs detected 129 (60,5%) patients and through the active screening 484 (39,5%) cases. From the total number of 213 cases 101 (47,4%) were confirmed by the positive GeneXpert MTB/RIF assay.

Demographic distribution established that in urban area of Chisinau city the new case incidence decreased (with 18,4/100.000 population) from 67,5/100.000 (487 patients) in 2011 to 49,1/100.000 (360 patients) in 2015. In rural area the new case incidence decreased more evidently from 162,0/100.000 (115 patients) to 75/100.000 (75 patients), thus showing a sharp downward trend (60,4/100.000 population). In this context, it is important to enumerate the surrounding villages included in rural area of Chisinau city in alphabetic order: Bacioi, Bic, Bubuieci, Budesti, Cheltuitori, Ciorescu, Codru, Colonița, Condrita, Cricova, Cruzesti, Dobrogea, Dumbrava, Durlesti, Fauresti, Frumusica, Ghidighici, Goian, Gratiesti, Hulboaca, Humulesti, Revaca, Stauceni, Strainsteni, Singera, Tohatin, Truseneni, Vadul lui Voda, Vatra, Vaduleni.

Multiple causes were involved in that fast downward trend: 1. insufficient rate of active screening investigations of high risks groups (close contacts with infectious TB sources, HIV infected individuals, patients with pulmonary sequels, patients with immunosuppressive therapy such as corticosteroids, specialized treatment for rheumatoid arthritis or Chron's disease, persons with psychiatric diseases; 2. Low addressability of patients to PHCP and

high rate of inaccessible patients [10]. Data are shown in the table 1.

Sex distribution established the predominance of men in comparison with women in both groups 48 (65.7%) in the 1st group and 22 (78.5%) cases in the 2nd group, with male/female ratio=1,92/1 in the 1st group and 3,67/1 in the 2nd group. Repartition of the patients into age groups according to the WHO recommendations, identified that the largest represented was 35-44 years old age group: 25 (34.2%) patients in the 1st group and 8 (28.6%) cases in the 2nd group, followed by the 45-54 years group 7 (25.0%) case in the 2nd group and 25-34 year group 18 (24.6%) patients in the 1st group. While redistributing patients in two age groups (young and >45years) it was established the predominance of younger cases (55 (75.3%)) in the 1st group comparing with 17 (60.2%) patients in the 2nd group, and older patients in the 2nd group 11 (39.3%) comparing with 18 (24.6%) in the 1st group without achieving statistical threshold. So, stratifying patients according to the biological characteristics it was argued that men and older individuals (>45years) are diagnosed more frequently by the screening methods and young persons, as well as women are more frequently detected and diagnosed through passive way of screening as symptomatic patients (table 1).

Table 1

Distribution of patients according to the demographic factors

Demographic factors		SG, n=73	CG, n=28	P value
		n (%)	n (%)	
Sex	Men	48 (65,7)	22 (78,5)	>0,05
	Women	25 (34,3)	6 (21,4)	>0,05
Young groups	18-24 years	12 (16,4)	3 (10,7)	>0,05
	25 - 34 years	18 (24,6)	6 (21,4)	>0,05
	35-44 years	25 (34,2)	8 (28,6)	>0,05
Older groups	45-54 years	13 (17,8)	7 (25,0)	>0,05
	>55years	5 (6,9)	4 (14,3)	>0,05
Residence	Urban	56 (76,7)	23 (82,1)	>0,05
	Rural	17 (23,4)	5 (17,9)	>0,05

Demographic distribution identified that all the enrolled patients were by origin from the Republic of Moldova, and in both groups there was a similar proportion of individuals from the urban and rural areas. Two thirds of patients were from urban area, and one third – from rural area. No homeless patients were identified among the selected cases. By distributing the patients according to the educational level, it was determined that individuals with low level of school education (primary and incomplete secondary school) were identified in a similar proportion in the 1st group and in the 2nd group: 21 (28.8%) and 7 (25%) cases, respectively. By distributing the patients according to the socioeconomic status, it was established that the rate of patients with economic stability was higher

in the 2nd than in the 1st group. So, one third of patients was employed in the 2nd group 10 (35.7%) patients comparing with 16 (21.9%) cases - in the 1st group. One half of both groups, (42 (57.53%) patients in 1st group and 13 (46.4%) patients in 2nd group) were unemployed. The totality of patients with low financial income (unemployed, retired and students) was in a similar proportion in both groups: 57 (78.1%) in the 1st group vs 19 (67.9%) cases in the 2nd group. Assessing the civil status it was identified the same rate of married persons in both groups: the 1st group (46 (63.1%) cases and 13 (46.4%) cases in the 2nd group. Patients living under the poverty line, with the income less than the minimum consumer basket were more frequently identified in the 1st group than in the 2nd group. Harmful social habits such as chronic alcoholism and drug use, as well as the history of imprisonment and migration during last year were identified in a small number of cases, without statistical differences between the groups. Epidemiological risk factor, such as a close contact and being a member of the family TB cluster statistically prevailed in the 1st group 17 (23,3%) vs. the 2nd group 2 (7.1%) cases, although the active way of detection is oriented especially to close contacts. So, it was identified the unsatisfactory use of active way of TB screening in infectious clusters (table 2).

Table 2

Distribution of patients according to the socio-economic factors

Demographic factors		SG, n=73	CG, n=28	P value
		n (%)	n (%)	
Educational status	Low (primary/incomplete secondary)	21 (28,8)	7 (25,0)	>0,05
	Secondary	30 (41,09)	14 (50,0)	>0,05
	Professional	14 (19,2)	4 (14,3)	>0,05
	Superior	8 (10,9)	1 (3,5)	>0,05
Economical status	Employed	16 (21,92)	10 (35,7)	>0,05
	Unemployed	42 (57,53)	13 (46,4)	>0,05
	Disabled	1 (1,37)	0	>0,05
	Student	8 (10,9)	2 (7,1)	>0,05
	Retired	6 (8,2)	4 (14,3)	>0,05
Social factors	Under minimum standard life	27 (36,9)	14 (50,0)	>0,05
	Migration	10 (13,67)	2 (7,1)	>0,05
	Alcohol abuse	2 (2,74)	1 (3,5)	>0,05
	Drug use	1 (1,37)	1 (3,5)	>0,05
	History of imprisonment	1 (1,37)	2 (7,1)	>0,05
	Family cluster of TB	17 (23,29)	2 (7,1)	<0,05

The rate of health uninsured patients was similar in both groups, as well as the rate of patients with comorbidities. So, two thirds of both groups were uninsured. One fifth of both groups were comorbid patients (figure 1). Delayed diagnosis due to the insidious onset of the disease

lasting more than 60 days statistically prevailed in the 1st group, 64 (87.7%) vs. 8 (28.6%) cases in the 2nd group. Summing up all patients that form high risk groups it was established that persons with risk for TB predominated in the 1st group 27 (36.9%) vs. 6 (21.1%) patients from the 2nd group. So, it can be concluded that passive way of screening contributes to delayed detection, diagnosis and treatment onset. On the other hand the low rate of patients from high risk groups detected through the active way of screening demonstrates poor disease control performed by PHCP in the frame of high risk groups (table 3).

Table 3

Case-management of TB patients and high risk groups

Characteristics		SG, n=73	CG, n=28	P value
		n (%)	n (%)	
Case management	Lack of health insurance	47 (64,4)	17 (60,7)	>0,05
	Associated diseases	17 (22,3)	5 (17,6)	>0,05
	Late detected (>60 days)	64 (87,67)	8 (28,57)	<0,001
	High risk groups	27 (36,9)	6 (21,4)	<0,05
High risk groups		SG, n=27 n(%)	CG, n=6 n(%)	
	HIV coinfectd	8 (29,6)	2 (7,14)	<0,001
	TB-contact	17 (62,9)	2 (7,14)	<0,001
	Immune suppressive treatment	1 (3,7)	2 (7,14)	>0,05
	Psychiatric disease	1 (3,7)	0	>0,05

High risk groups were designed to be examined compulsorily annually and free of charge. Despite this recommendation, the rate of such individuals was statistically higher in the 1st group, 27 (36.9%) cases compared to the 2nd group – 6 (21.4%) cases. Being one of subgroups, HIV-infected individuals were more frequently in the 1st group 8 (29.6%) comparing with the 2nd group 2 (7.14%) cases. More evidently, TB contacts were more frequently identified in the 1st group - 17 (62.9%) cases comparing with the 2nd group of 2 (7.14%) patients. There were no differences in the rate of patients with immune suppressive treatment, and psychiatric diseases identified in both groups.

When assessing the laboratory features of the enrolled new pulmonary TB cases, it was identified that one half of patients was microscopic positive for acid-fast-bacilli in the 1st group and only one third - in the 2nd group. The same rate was identified in the repartition of patients according to the positive bacteriological results (culture on solid Lowenstein-Jensen ether liquid MGIT BACTEC). The sensibility to the rifampicine through GeneXpert MTB/Rif assay was established more frequently in the 1st group than in the 2nd group, without achieving the statistical threshold (table 3).

Assessing radiological features of investigated patients it was established a high degree of difference between the

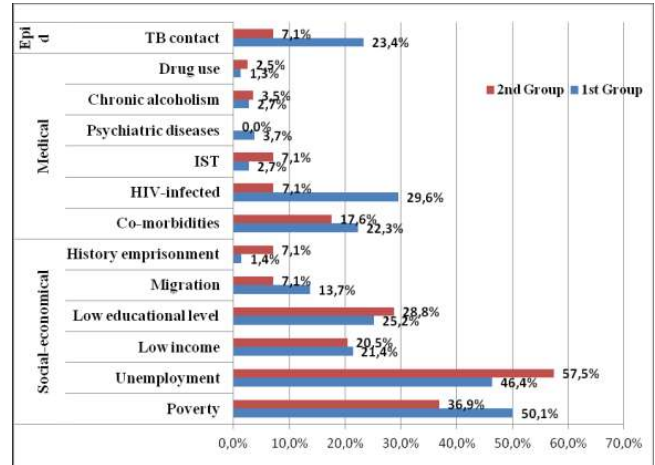


Fig. 1. Risk factors for active disease development.

Note: IST-immune suppressive treatment.

groups regarding the extensibility of infiltrative processes. More frequently were patients with 1 lung involved in the 2nd group, 26 (92.8%), and with both lungs affected in the 1st group, 63 (86,3%) cases. Lung infiltrates were complicated with destructions in two thirds of the 1st group, 49 (67,1%), and only in a couple of patients from the 2nd group (table 4). This fact is due to the late detection of new TB cases in the 1st group and earlier detection in the 2nd group.

Table 4

Microbiological, radiological features and treatment outcome

Characteristics		SG, n=73	CG, n=28	P value
		n (%)	n (%)	
Microbiological	Microscopic positive	38 (52,01)	10 (35,7)	<0,001
	Culture positive	40 (54,8)	11 (39,3)	>0,05
	GeneXpert MTB/Rif sensible	47 (64,4)	20 (71,4)	>0,05
	GeneXpert MTB/Rif resistant	26 (35,6)	8 (28,6)	>0,05
Radio-logical	1 lung involved	10 (13,7)	26 (92,8)	<0,001
	2 lungs involved	63 (86,3)	2 (7,14)	<0,001
	Lung destructions	49 (67,1)	2 (7,14)	<0,001
	Infiltrative TB	67 (91,8)	25 (89,2)	>0,05
	Disseminated TB	6 (8,2)	3 (10,7)	>0,05
Treatment outcome	Healed	30 (41,09)	10 (35,7)	>0,05
	Treatment completed	8 (10,9)	5 (17,85)	>0,05
	Dead	3 (4,1)	0	>0,05
	Continuing TB treatment (individualized regimen)	0	13 (46,24)	
	Continuing DOTS plus treatment	16 (21,9)	5 (17,8)	>0,05
	Hospitalized during intensive phase	73 (100)	28 (100)	>0,05

All patients were treated during intensive phase in the Chisinau Municipal Hospital of Pneumophthysiology. By

assessing the final treatment outcome, it was identified a similar rate of healed patients treated with standard regimen for new TB case in both groups, 30 (41.1%) cases in the 1st group and 10 (35.7%) cases in the 2nd group. As well as, completed the specific treatment a similar proportion of patients in both groups, 8 (10.9%) cases in the 1st group comparing with 5 (17.85%) cases in the 2nd group. Died only 3 (4.1%) patients from the 1st group, comparing with no registered deaths in the 2nd group. Almost one half of patients (13 (46.24%)) from the 2nd group are still continuing an individualized regimen. A similar rate of patients from both groups is performing the treatment for MDR-TB.

Conclusions

In 2015, in Chisinau, primary health care providers identified only 101 new pulmonary TB cases with positive GeneXpert MTB/RIF assay from a total number of 213 cases, 78 cases as symptomatic patients and only 23 patients by active screening.

By distributing patients according to the sex, it was identified the predominance of men in comparison with women in both groups, but the male/female ratio was 2 times higher in the group detected by active screening.

All patients were by origin from the Republic of Moldova. No homelessness was registered. Two thirds of patients were from urban area of Chisinau. Data confirm the inaccessible primary health care for immigrants, homeless and low accessibility for rural population.

Educational and civil status was similar in both groups of selected patients.

Economic status was higher in the group detected by active screening. On the other hand the unemployment and rate of patients living under the poverty line were higher in the group of patients detected as symptomatic cases.

Two thirds of patients from both groups were uninsured, demonstrating their low accessibility to health care.

Chronic alcoholism, drug use, history of imprisonment, migration were established in a small number of cases from both groups. Co-morbidities were identified more frequently in the symptomatic group of patients.

Close contact with infectious source, as well as HIV infection prevailed in the group detected as symptomatic, despite the fact that those patients might have been detected by active screening.

High statistical difference between groups was established comparing microbiological and radiological features. Positive bacillary state, extensive process and lung destruction were more frequent in the group of symptomatic patients.

The same rate of successfully finished the treatment in both groups contrasted with death rate registered only in the groups of symptomatic patients.

In overall, the Republic of Moldova registers a continuous decrease of epidemiological TB indices due to the reduction of high risk groups designed to be investigated and regretfully to insufficient use of active screening.

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Inflammatory pathologies of genitalia in girls and adolescents

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Abstract

Background: Children and adolescents are a group of population that represents 1/3 of territorial population with specific biological peculiarities of age, activity, health and morbidity. Professional medical assistance given to the young generation ensures for subsequent generations a nice and healthy future.

Material and methods: The present study is non-experimental, of a descriptive type with a general group of 400 patients hospitalized during 2014 in the department of pediatric gynecology. Statistical processing was performed using the program «Epiinfo 2002» and the program «Microsoft Office Excel 2010».

Results: According to the obtained results within the study the inflammatory diseases of genitals were most frequently diagnosed – 263 cases (65.8±2.37%), the majority of which 254 (96.6±1.11%) of a non-specific genesis and 9 cases (3.4±1.11%) bacterial infections of specific genesis, P<0.001. In half of the investigated cases 108 (52.7±3.48%) there have been cultivated combinations of pathogens, the most frequently determined being: *E. coli+corinebacterium*, *E. coli+corinebacterium +Enterococcus faecalis*, or *Corinebacterium + St. Viridans*, *Streptococcus saprophyticus+corinebacterium*. From the total number of non-specific inflammatory diseases prevail inflammatory diseases of external and inferior internal genitals 169 (66.5±2.96%), P<0.001 – vulvovaginitis: acute vulvovaginitis, chronic relapsing vulvovaginitis with a maximum share in the age group 1-4 years old 68 (40.2±3.79%). Contrary to the distribution of inflammatory diseases of the inferior and external genital tract, the inflammatory diseases of the upper genitals increase with the advancement in age of the patients involved in the study with a maximum rate in the group of adolescents of 16-19 years old (55.4±5.47%), P<0.001.

Conclusions: An increase of the inflammatory processes of genitals in the age groups 1-4 (22.3%) and 16-19 (25.8%) was determined. A maximum share of inflammatory diseases of inferior genitals was determined in patients aged 1-7 and inflammatory diseases of the upper genital tract in the period of adolescence – 14-19 years old.

Key words: girls, adolescents, inflammatory process.

Introduction

Children are considered to be an investment of parents, a population of reproductive age, of the country in the future of the society. A good, satisfactory state of health of the girls and adolescents beginning with the antenatal period, maintained and consolidated in childhood and adolescence, constitutes an important resource for the health of future generations. The health of girls and adolescents should be one of the major concerns for policy makers because health insurance at this age is a guarantee of an acceptable level of health for generations to come [1,2,3]. Unfortunately, maintaining and strengthening the health of the general population, including girls and adolescents in the Republic of Moldova today is difficult due to the mass migration of the population, including the female population until adolescence; poor economic condition of the country and poor living conditions, especially in rural areas; low education level of the population. These factors influence the increase in infantile gynecological morbidity which largely affects their sexual development, their reproductive function and the decreasing of social activity [4]. According to some reports a decrease in the general health of girls during the last 10 years from 28.6 to 6.3% is observed [5].

The structure of gynecological morbidity in girls varies according to age and stages of their body development [6, 7]. If we analyse the distribution of gynecological diseases in the general structure of gynecological morbidity, then it can be observed that it does not vary in different regions

and countries of the world, the inflammatory diseases of the genitals having the highest frequency (65.8-63%).

The aim of the study: Evaluation of incidence and distribution of inflammatory diseases of genitals in girls and adolescents from the study in different life periods.

Objectives: 1. Determination of cardinal clinical symptoms of inflammatory diseases of genitals in patients from the study; 2. Evaluation of laboratory parameters, instrumental in patients with inflammatory diseases in the studied group; 3. Analysis of reference diagnostics, clinical admission and final diagnoses of the patients from the study; 4. Distribution of inflammatory diseases of genitals in patients from the study according to their age.

Material and methods

The present study is non-experimental, of a descriptive type with a general group of 400 patients hospitalized during 2014 in the department of pediatric gynecology of the National Institute of Mather and Child, Chisinau, the Republic of Moldova. Methods of data collection in the study were based on extraction of medical documentation data from archive to complete the questionnaire for research. Statistical processing was performed using the program “Epiinfo 2002” and the “Microsoft Office Excel 2010”.

Results and discussion

Analyzing the obtained data it was found that from the age of 3 weeks to 19 years old the average age of patients studied was 11.4 ± 1.26 years old (fig. 1).

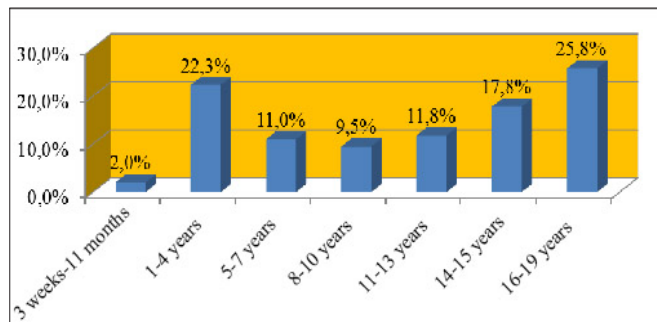


Fig. 1. Distribution of the patients in the study according to their age.

By place of residence 205 patients come from the urban area (51.3±2.49%) and 195 (48.7±2.49%) from rural area (table 1).

Table 1

Distribution of patients according the place of residence

No	Place of residence	Abs. n=400	P±ES (%)	t	P
1	Urban	205	51.3±2.49	0,7356	>0.05
2	Rural	195	48.7±2.49		

Performing a comparison between reference diagnostics and established diagnoses at admission it was found that in reference diagnostics of the patients in the hospital predominate the inflammatory pathologies of the genital organs 250 (62.5%): 244 (97.6%) non-specific inflammatory diseases and in 6 (2.4%) cases inflammation of the genitals of specific origin (urogenital mycoplasmosis, genital herpes, warts acuminata). From the total number (244) of clinical cases with non-specific inflammatory diseases 180 (73.8%) are attributed to inflammation of external and internal inferior genital organs (acute vulvovaginitis 98 (54.5%); recurrent vulvovaginitis 80 (44.4%); acute Bartholin's 2(1.1%)), and 64 cases (26.2%) inflammatory pathologies of internal superior genital organs (acute salpingoophoritis 41 (64%), subacute salpingoophoritis 18 (28.2%), chronic recurrent salpingoophoritis 5 (7.8%). In diagnoses, established at admission, dominate the same categories of pathologies without major differences (table 2).

Table 2

Distribution of gynecological diseases according to reference and hospitalisation diagnoses

Gynecological maladies	Reference diagnostics n =400		Admission diagnostics n =400	
	Abs	P±ES	Abs	P±ES
Inflammatory pathologies of genital organs	250	62.5±2.41	251	62.7±2.41

After evaluating the clinical picture of inflammatory processes of genitalia in girls and adolescents there were highlighted most common symptoms: pain in 140 cases (35 ± 2.38%), P <0.001; pathological leucorrhoea in 210 cases (52.5 ± 2.49%), P <0.001; itching genitals – 102 cases (25.5 ± 2.18%), P <0.001. The extragenital complaints are attributed mainly to urinary tract damage, dysuria 19 (4.75±1.06%), P<0,001, fully associated with inflammatory diseases of the external genitalia.

Depending on the onset of the inflammatory process: acute onset / primary was determined in 239 cases (59.8 ± 2.45%), chronic evolution, relapsing diseases in 161 cases (40.2 ± 2.45%), p <0.001 (fig. 2).

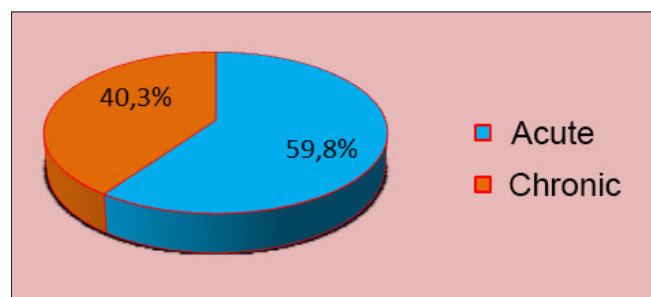


Fig. 2. Graphic representation of patients according to onset of disease.

Most patients in the study 264 (66.0 ± 2.36%) were hospitalized in satisfactory, at 121 patients (30.3 ± 2.29%) was established a state of medium severity and 15 patients (3.8 ± 0.95%) were hospitalized in serious condition.

On physical examination there was found hyperemia of vulvar mucosa in 260 cases (65.0 ± 2.38%). Vulva swelling was recorded in 234 cases (58.5 ± 2.46%), accompanying entirely the mucosal hyperemia, P <0.001. Purulent discharges from the genital tracts are found in 144 cases (36.0 ± 2.40%), bloody deletions in 72 cases (18.0 ± 1.92%) and only 3 cases (0.8 ± 0.44%) with cheesy discharges.

At gynecological examination of genitals (exam with specula; vagino-abdominal/recto-abdominal exam; vaginoscopy) performed in 154 cases (73.3 ± 3.05%) there were determined modifications, P <0.001, of which on the first place is: sensitive annexes in 127 cases (59.6%), hypoplastic uterus was found in 1 case (7.8%), ovarian enlargement in size in 13 cases (8.4%), the presence of the foreign body in the vagina and hypoplastic ovaries were determined in 2 cases (0.9%), rudimentary uterus in one case (0.5%).

The patients in the study who were hospitalized in the department of pediatric gynecology had undergone a set of laboratory investigations. Leukocytosis with a drift to the left has been detected in 75 cases (18.8±1.95%), identified in patients with acute inflammatory process (acute salpingoophoritis, subacute salpingoophoritis, chronic recurrent salpingoophoritis) of the superior genitalia – 34 (45.3±5.75%), leukocytosis less characteristic for the lower genital inflammation – 12 (16.0±4.23%), in 10 cases

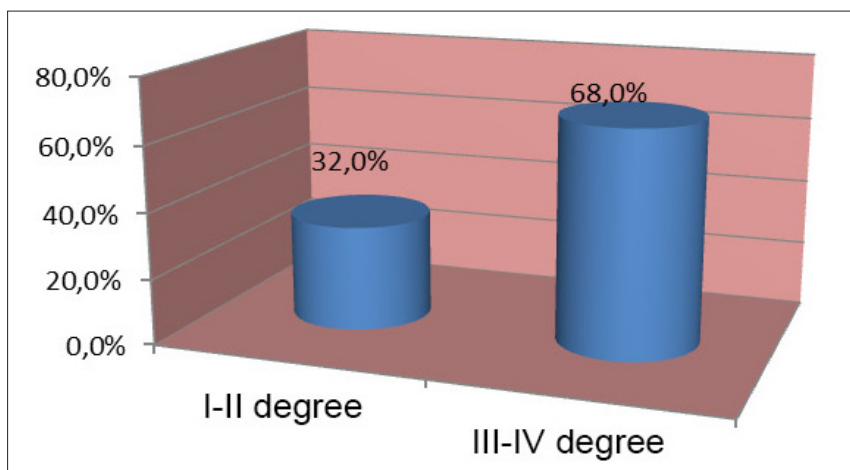


Fig. 3. Results of bacterioscopic exam in patients of the study.

(13.3±3.92%) leukocytosis was identified in patients with other complications (foreign bodies 3; trauma of genitalia 1; ovarian cysts 6).

It should be mentioned that leukocytosis is not a specific criterion only for inflammatory diseases of genitalia because the rest of the patients 19 (25.3±5.01) with leukocytosis within the study had other sources of bacterial infections in the body 7 (36.8%) or formed a combination with inflammatory diseases of genitalia 12 (63.2%) (e. g. vulvovaginitis + acute cystitis; vulvovaginitis + chronic recurrent/acute pielonefritis; inflammatory diseases of the annexes of uterus + chronic/acute pielonefritis, chronic cystitis; inflammatory diseases of external/internal superior genitalia associated with bacterial infections of upper respiratory tract). Eosinophils increase in the general analysis of blood marks the girls' contact with various parasites (ascariasis, toxocarosis, giardiasis, lamblia, etc).

Bacterioscopic exam results indicate the presence of inflammatory process of genitals (purity grade III-IV) in 272 patients (68.0 ± 2.33%) in the study group, $P < 0.001$ (fig. 3).

The bacterioscopic exam (table 3) was followed by bacteriologic investigations in 205 cases (75.4±2.60%), biological material was taken from the vagina 191 (97.5±0.78%), vulva 6 (2.0±0.70%), urine 3 (0.5±0.35%). In half of the investigated cases 108 (52.7±3.48%) were cultured combinations of pathogens, the most frequently determined were: *E.coli+corinebacterium*, *E.coli+corinebacterium+Enterococcus faecalis*, or *Corinebacterium + St. Viridans*, *Streptococcus saprophyticus+corinebacterium*. Below in table 3 the rate of pathogens according to their detection in patients is presented. Thus, the most frequent increase is attributed to optional microbes: *Corynebacterium spp.* 96 (46.8%), followed by *E.coli* 63 (30.5%), *Enterococcus faecalis* 50 (24.4%), *Stafilococcus aureus* 20 (9.8%), *Str. viridans* 17 (8.3%), *Str. saprophyticus* 15 (7.3%), *Staph. epidermidis* 14 (6.8%), *Klebsiella pneumoniae* 12 (5.9%), etc. It is to be mentioned that an increase of the anaerobic agents,

known as the most aggressive, was not found in patients who undertook the bacteriological exam. Rarely detected were: *Proteus vulgaris* 1 (0.5%); *Proteus mirabilis* 3 (1.5%); *Ps. Aerogenosa* 3 (1.5%), *Hemophilus influenzae* 2 (1%), *Gardenerlla vaginalis* 2 (1%), *Klebsiella oxytoca* 7(3.4%), *Candida glabrata* 1 (0.5%), *Candida krusei* 1 (0.5%).

Serological exams with the purpose to determine the infections and their evolution were carried out in 121 patients (30.3±2.29%). Immune antibodies (IgA, IgM, IgG) were identified in 48 cases (39.7±4.44%): *Mycoplasma hominis* 30 (62.5±6.98%) (in 28 cases there was determined a chronic infection, Ig G being detected in 2 cases – recent infection marked by positive titres of IgM and IgA); *Ureaplasma urealiticum* 17 (35.4±6.90%) (chronic infection in 14 cases, marked by positive titres IgG and 2 cases of fresh infection, marked by the presence of IgM; IgA); *Ascaris lumbricoides* detected in 17(14.6±5.09%) (6 patients with a history of infection, marked only by titres IgG and 1 case with a recent infection with positive IgA titres); chronic

Table 3

Ranking of pathogens within the bacteriological exam in patients from the study

No	Pathogens	N=205	P (%)
1	Corynebacterium	96	46.8
2	E. Coli	63	30.5
3	Enterococcus faecalis	50	24.4
4	Stafilococcus aureus	20	9.8
5	Streptococcus viridans	17	8.3
6	Streptococcus saprophyticus	15	7.3
7	Klepsiella pneumonia	12	5.9
8	Staphylococcus epidermitis	14	6.8
9	Candida albicans	9	4.4
10	Streptococcus β-hemolitic	7	3.4

infection with *Clamidia trachomatis* 3 (6.3±3.50%) (Positive IgG titres); *Toxocara catis/canis* 4 (8.3±3.98%) (3 cases of chronic infection – IgG and 1 case of acute infection, marked by positive IgA); *Giardia lamblia* 4 (8.3±3.98%) (in 3 cases chronic parasitosis/IgG “+” and 1 case of acute infection/IgA “+”).

Thus, the laboratory serological results allowed us to find out the contact of the patients with the above mentioned pathogens which can be the cause of the development of the inflammatory diseases of the upper internal genitalia as well as the external genitalia in case of parasitic diseases. It was not possible to determine this «link» in patients from the study because of dispersing/discordance of the obtained results in analysis and interpretation of the laboratory parametres mentioned above.

Full assessment, full division and ranking of gynecological diseases; division and distribution of gynecological diseases according to the age of girls and adolescents in the study group was performed after an analysis of conclusive clinical diagnostics at discharging patients from the hospital.

The first place in the ranking of gynecological diseases in girls and adolescents in the study was attributed to inflammatory pathologies of genitalia, diagnosed in 263 cases (65.8±2.37%): the majority 254 (96.6±1.11%) of a non-specific genesis and 9 (3.4±1.11%) bacterial infections of specific genesis (8 urogenital mycoplasmosis, 1 urogenital ureoplasmosis), P<0.001. From the total number of non-specific inflammatory diseases the inflammatory diseases of external and internal inferior genitalia prevail 169 (66.5±2.96%), P<0.001 – vulvovaginitis: acute vulvovaginitis 135 (79.9±3.08%); chronic relapsing vulvovaginitis 33 (19.5±3.04%); acute bartholinitis 1(0.6±0.59%). Non-specific inflammatory diseases of the upper internal genitalia were diagnosed in 183 patients (32.7±2.94%), found

as salpingoophorites in various phases of evolution: acute salpingoophoritis 68 (81.9±4.22%), subacute salpingoophoritis 11 (13.3±3.72%), chronic relapsing salpingoophoritis 4 (4.8±2.34%) (table 4).

Table 4

General division of gynecological diseases in patients from the study

Gynecological diseases	Clinical diagnostics n=400		Dignostics at hospitaliaza-tion n=400		t	P
	Abs	P±ES	Abs	P±ES		
Inflammatory pathologies of genitalia	263	65.8±2.37	251	62.7±2.41	0.9198	>0.05

Another aspect which we had proposed ourselves to analyse was the general and separate distribution of various gynecological diseases mentioned above in different age groups. The analysis of the group of inflammatory diseases of genitalia (fig. 4) showed the predomination of non-specific inflammatory pathologies of external and inferior internal genitalia in age categories between 1-4 years old 68 (40.2±3.79%), P<0.001, with a diminishing rate when the girls advance in age. Thus, according to the graphic representation below, an obvious decrease in the rate of inflammatory diseases of external and inferior internal genitalia from 5 to 15 years old is observed, with a tendency to increase inflammatory processes in late adolescence 16-19 years old (5.3±1.73%), P>0.05. On the contrary, the distribution of inflammatory diseases of lower and external genital tract, inflammatory diseases of the upper genitalia increase with the age of the patients involved in the study, with the maximum rate in the group of 16-19 years old 46 (55.4±5.47%), P<0.001.

Following the presentation of data on distribution of

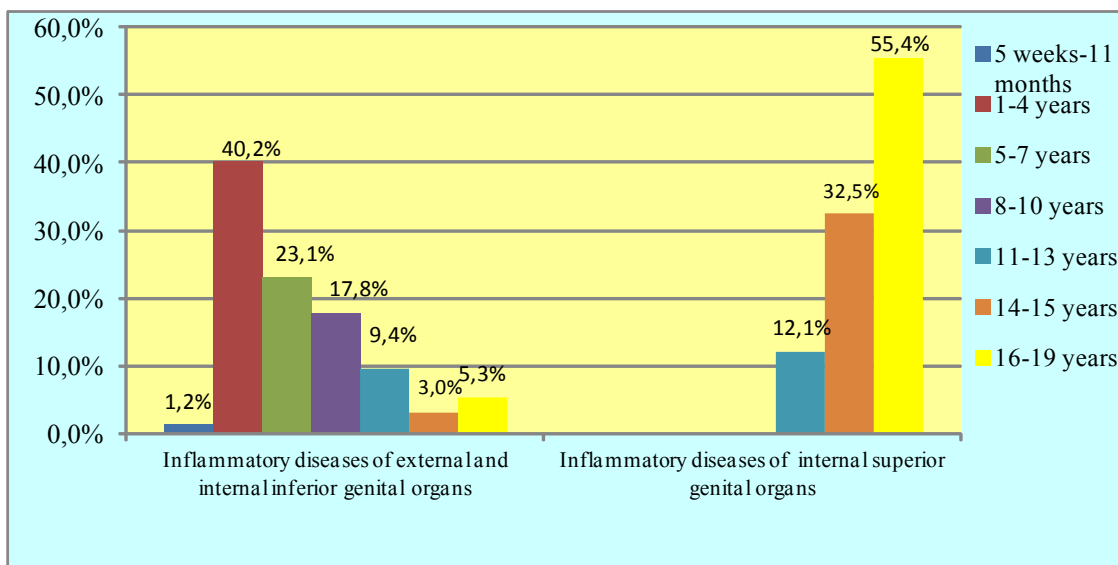


Fig. 4. Division of inflammatory diseases of genitalia in age groups.

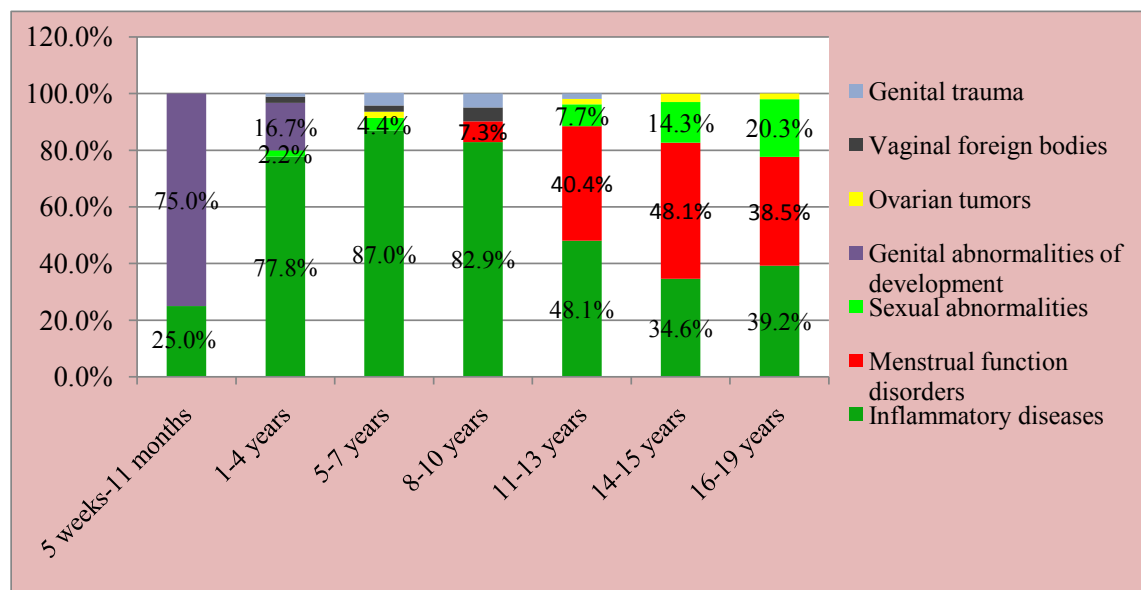


Fig. 5. Distribution and classification of gynecological diseases in various age groups.

inflammatory diseases of the genital organs, it can be concluded that vulvovaginitis occurs frequently during 1-4 years old, explained by poor hygiene in this group, helminthiasis was determined only in this age group, peculiarities of the female gonadostat functioning at this age [8,9]. Other causes cannot be mentioned because of the lack of data in the studied medical documents. Surprising were the results of determining an increased rate of inflammatory diseases of the upper genital tract in adolescents although there was no evidence, facts in medical records that would reveal the cause of their appearance (e.g. sexually transmitted diseases, manipulations of genitalia, presence of other extragenital outbreaks of infection) [7,10].

Thus, in the group of girls at the age between 5 weeks and 11 months predominated anomalies of external genital organs (labial agglutination) 6 (75±15.30%) and only in 2 cases (25±15.30%) – vulvovaginitis.

Graphic representation of gynecological diseases in patients of the age group 1-4 years old demonstrates an increased frequency of inflammatory diseases of external and internal genital organs – vulvovaginitis 70 (77.8±4.38%); in 68 cases (97.1%) and 2 (2.9%) cases of specific inflammations (mycoplasmosis and chronic urogenital ureoplasmosis), followed by anomalies of genital organs – synechiae of pudental labia 15 cases (16,7±3.93%). Other gynecological diseases in the group 1-4 years old were distributed in the following way: trauma of the external genitalia 1 (1.1%), abnormalities of sexual development 2 (2.2±1.54%) presented by early telarche and virile syndrome; foreign bodies in vagina 2 (2.2±1.54%).

The period of life aged 5-7 years old is represented by inflammatory pathology of genital organs 40 (87±7.22%). The rest of gynecological pathologies in this group is attributed to the trauma of external genital organs 2 (4.3±2.99%); abnormalities of sexual development 2 (4.3±2.99%), mani-

festated by early telarche and virile syndrome of an unknown etiology; isolated cases of foreign body (2.2±2.15%) and ovarian cyst (2.2±2.15%).

In the age group 8-10 years old the inflammatory pathologies of genitals remain in the top 34 diseases (89.5 ± 5.96%) with predominance of non-specific inflammations of the external and inferior internal genitalia 30 (88.2%) compared with inflammatory diseases of specific origin 4 (11.8%). Other diseases in this group are uterine bleeding (dysfunctional bleeding) 3 (7.9%) in patients with early menarche; complications connected with foreign bodies 2 (5.3%) and traumas of the external genitalia 2 (5.3%).

The pathologies specific to patients aged 11-13 do not change their status, inflammatory pathologies of genitalia being on the first place 25 (48.1±6.92 %) with predominance of the inflammation of the lower genital tract – vulvovaginitis 15 (60%) and 10 cases (40%) of inflammation of the upper genital tract which occurred primarily in the structure of inflammatory diseases in this age group.

During middle adolescence of 14-15 years old the inflammatory pathology of genitals is followed by menstrual dysfunction within the ranking of this group 36 (35.5±4.69%), with predominance of inflammation of upper internal genitalia 27 (75%) and there was determined a clear decrease of the inflammation of inferior genitalia 5 (13.9%).

Almost equally are distributed the groups of inflammatory diseases of the genitals 57 (38.5±4.08%) and diseases accompanied by disorders of the menstrual function 58 (39.2±4.09%) in adolescents of 16-19 years old. The structure of the inflammatory infections is constituted of the maximum rate of the inflammatory diseases of the upper genital tract 46 (80.7%), with a decrease of inflammatory diseases of the inferior genital tract 9 (15.8%) (fig. 5).

Thus, the analysis of clinical diagnostics of distribution

of gynecological diseases according to age and the general distribution of gynecological diseases gives us the possibility to create a structure of gynecological morbidity in the framework of the present study. The structure and ranking of gynecological diseases is constituted of inflammatory diseases of genital organs, diseases accompanied by menstrual disfunction, abnormalities of sexual development, anomalies of genitals and tumors of the genitals. The represented structure is similar to the structure of gynecological morbidity in different countries inclusively the Republic of Moldova [3,11,12].

Conclusions

1. The inflammatory pathology of genitals occupies the main place ($65.8 \pm 2.37\%$) in the structure of gynecological morbidity in patients from the study;

2. The following is informative for the diagnosis of inflammatory diseases of genitals: bacterioscopic and bacteriologic examination of samplings from genitals, the vulvar prints; vaginoscopy; the coprologic examination to helminths; serological examination to various parasites and specific infections;

3. Pain ($35 \pm 2.38\%$); pathological leucorea ($52.5 \pm 2.49\%$); genitals itching ($25.5 \pm 2.18\%$) and disurea 19 ($4.75\% \pm 1.06\%$) constituted the clinical picture with the most frequent symptoms observed in patients of the studied group;

4. There was determined an increase of the inflammatory processes of genitals in the age groups 1-4 (22.3%) and 16-19 (25.8%);

5. As the most frequent etiological factors in the non-specific inflammatory processes in the majority of age groups there were determined facultative microbes: *Corynebacterium spp.* 96 (46.8); *E.coli* 63 (30.5%); *Enterococcus faecalis* 50 (24.4%); *Stafilococcus aureus* 20 (9.8%); *Str. viridans* 17 (8.3%); *Str. saprofiticus* 15 (7.3%);

6. A maximum share of the inflammatory diseases of the inferior genitals was determined in patients at the age between 1-7 years old and the inflammatory diseases of the genital tract in the period of adolescence 14-19 years old.

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Indoor air quality assessment and the risk establishment for the health of the people engaged in the process of preparing food

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Abstract

Background: The health state assessment of the inhabitants of the Republic of Moldova in relation to the pollution of the indoor air during the process of preparing food.

Material and methods: The study was conducted in three areas of the Republic of Moldova (North, Center and South). The research was based on the inhabitants' inquiry and some measurements of physical parameters (the temperature, the relative humidity of the air) and some chemical compounds from the air (the carbon dioxide and the carbon monoxide) from the air. The measurements were carried out during summertime, with Air Quality Monitor 500 in 4 stages: before cooking meals, one hour after the beginning of cooking, at the end of the cooking and one hour after the end of preparing dishes. During the research a questionnaire has been completed, which included a number of questions referring the conditions present in the process of cooking meals, the time needed for cooking, the illnesses supported, the type of the cooking appliances, the type of the fuel used.

Results: The analysis of the questionnaires emphasized that half of the respondents have suffered frequently from bronchitis, 28% have been sick with pneumonia, 12% of the people suffered from obstructive bronchitis, 7% – from bronchial asthma and one person – from lung cancer. From the number of the respondents inquired 20% presented a diagnosis of ischemic heart disease and 7% – a stroke. The type of the room in which the people surveyed cooked the meals represents a certain risk in the development of the diseases. There is a huge risk to prepare the dishes in the living room ($2.6 \leq RR \leq 2.9$) for different nosologic forms ($p < 0.01$). The relative risk of the appearance of the diseases rose depending of the fuel used: biomass ($3.3 \leq RR \leq 4.5$, $p < 0.05$), agricultural waste ($3.5 \leq RR \leq 3.6$, $p < 0.01$), hard coal ($3.4 \leq RR \leq 3.5$, $p < 0.01$), in comparison to other types of fuel. In cases when the interviewees smoke in the house, the risk is very high ($3.4 \leq RR \leq 5.2$, $p < 0.001$).

Conclusions: It is necessary to focus our efforts on the communication strategies in order to motivate the awareness of the need of educational programs intended to inform people about the sources of pollution of the rooms and the health risks.

Key words: indoor pollution, air, cooking, risk, health.

Introduction

According to a study of OMS [6, 7], 3.7 million people died because of the outdoor pollution effects and 4.3 million – as a result of air pollution in the households, that is: smoke and emissions associated with cooking appliances, the fuel used (wood or coal), or because of heating appliances. The most frequent diseases caused by air pollution are the lung diseases, heart diseases and cancer. The study from 2012 also showed that there is a stronger connection, much stronger than it was believed, between air pollution and heart diseases, as well as between lung cancer and air pollution.

Therefore, air pollution is one of the most important global risks of mortality and is to blame for the rise of the risk of suffering from chronic diseases [2, 5]. An important role in air pollution in cooking spaces is played by the type of fuel used. The greatest potential of indoor air pollution is attributed to peasant stoves, which function on such fuel as: wood, brushwood, biomass, agricultural waste, etc.

In rural areas biomass is frequently burnt in traditional stoves with an open fire, in poorly ventilated rooms with smoke emissions. The smoke emitted contains large quantities of pollutants, which lead to severe consequences for the people exposed to it. The study made in India showed that 4-6% of the national burden of the diseases are attributed to the use of solid fuel. The researches made about the

use of the fuel in Indian households and the epidemiologic studies of the risks of the indoor air pollution in a series of developing countries indicate that annually approximately 440.000 of premature deaths of children under 5 and 34.000 of female deaths resulted from chronic respiratory diseases, as well as 800 cases of lung cancer can be attributed to the use of solid fuel. The recent study carried out by the World Health Organization established the burden of the diseases slightly smaller in India for the year 2000 [1, 3].

The perceived level of air pollution and the risks associated with health, as the study of the community states, have been reduced among the inhabitants, thus indicating the necessity to grow the awareness of the sources of air pollution and the risks for health associated with them [2].

Material and methods

The study was conducted in three areas of the Republic of Moldova (north, center and south). The research was grounded on the questioning of the inhabitants and the measurements of some physical parameters (the temperature, the relative humidity of the air) and chemical compounds (the carbon dioxide and the carbon monoxide) from the air. The measurements have been made in all types of rooms where the population prepares meals: kitchens, living rooms, summer kitchens, etc.

The measurements were carried out during summer-

time, with Air Quality Monitor 500. 150 households were included in the study (50 from each geographical area). The stages of data collection were the following: before cooking meals (the I-st stage of measurements), in an hour after the beginning (the II-nd stage of measurements), at the end (the III-rd stage of measurements) of the cooking and an hour after the end of preparing dishes (the IV-th stage of measurements) during summertime. The questionnaire has been composed of 11 items and filled with the help of the method of direct interview. To evaluate the conditions of cooking meals, there have been asked questions about the place of cooking, the type of fuel used, in order to evaluate the influence of the cooking conditions on health, the respondents have been also asked about the diseases from which they suffered. To analyze the risk of the appearance of some diseases related to the type of room used for cooking, the type of fuel used, the presence/absence/functioning of the ventilation, the relative risk (RR), attributable (RA), and the attributable fraction (FA) have been calculated.

The relative risk shows how many times larger is the proportion of the people with certain modifications amongst the ones exposed to the risk factors in relation to the proportion of the people unexposed to the risk factors (tab. 1). The risk evaluation of the factors predisposing to cause the unwanted effects upon the state of health has been done on the basis of the "Table of contingency 2x2". The interpretation of the results for the relative risk in made in relation to 1.

Table 1

The interpretation of the results of the relative risk [4]

RR	Conclusions	RR	Conclusions
RR<1	Protection factor	0.0 – 0.3	Strong protection factor
		0.4 – 0.5	Moderate protection factor
		0.6 – 0.9	Reduced protection factor
RR=1	Indifferent factor	1.0 – 1.1	Indifferent factor
RR>1	Risk factor	1.2 – 1.6	Reduced risk
		1.7 – 2.5	Moderate risk
		>2.5	High risk

The *attributable risk* shows how much higher is the frequency of the unwanted effect upon the ones exposed, in relation to 0. The *attributable fraction* shows the percentage of how much the unwanted effect present at the people exposed is due to the risk factor. In order to establish whether there is a significant difference from the statistical point of view between the groups surveyed, the value of the calculated χ^2 has been compared to the one in the table. The χ^2 table test (with liberty degree=1, for the "Table of contingency 2x2", which has been used in evaluating the risk) presumes a probability of 0.05 at the value of 3.8; 0.01 probability at the value of 6.6 and 0.001 probability at the value of 10.8. If the probability (p) is smaller than the criti-

cal value with 0.05, then there is a significant difference between the cases observed and the ones expected, thus the null hypothesis is discarded. Any statistical analysis always includes in a bigger or smaller proportion a series of errors resulted from the sampling (the so-called effects of random sampling), therefore the value RR calculated from the data of the study will not probably be identical to the "real" value of RR. The statistical analysis gives us the possibility to determine the "real" value of RR with the help of the interval of confidence (CI) of 95%, which presumes that in 95% of the cases this interval includes the "real" value of RR. In order to interpret the results of the statistical analysis in a pertinent scientific context, both extremities of the interval of confidence should be analyzed [4].

Results and discussions

The research that has been fulfilled has shown that the indoor air is more polluted in the case of cooking food in the inhabited areas, that is, in the living rooms, thus influencing the state of health of the inhabitants. There has been concluded that even in the cases when the population had rooms used as kitchens, the cooking conditions were not favorable, not corresponding fully to the requirements. When preparing meals in summer kitchens, the chemical and physical parameters of the air were relatively closer to normal values because the respective rooms communicate directly with the outside air. The values of the parameters studied also depend on the type of cooking installations used by the population and the fuel used. The research has shown that the air was more polluted in the case of the usage of peasant stoves. The bottled gas used for the cooker polluted the air even more than the natural gas. Among the types of fuel used in peasant stoves more pollutant were: agricultural waste, biomass, hard coal.

For the purpose of estimating the risk of contracting certain diseases that depend on the type of space used for preparing dishes, the type of cooking appliances, the type of fuel used, the presence/absence/functioning of the ventilation, the possibility to open the windows for airing, the duration and frequency of cooking, the smoking, the values of physical and chemical factors present in the spaces used for cooking, there have been calculated: the relative risk, the attributable risk and the attributable fraction. The evaluation of the role of the cooking conditions, the environmental factors in these rooms, through the calculation of the relative and attributable risk, has the purpose to emphasize their prevalence, their hierarchical place and degree of influence, in order to guide us toward the elaboration of a set of measures to reduce their negative impact.

The type of room, where people prepare food presents a certain risk in the disease development. Due to cooking in the living room, there is a high risk ($2.6 \leq RR \leq 2.9$) to contract different nosologic forms ($p < 0.01$). 28.3 times ($p < 0.01$) are more likely to risk people preparing food in the living room than in kitchens and 21.7 times ($p < 0.01$) more than in summer kitchens. In the case of preparing food in

kitchens, the risk is moderate ($1.8 \leq RR \leq 1.9$, $p < 0.01$) and the population risks 12.4 times more than those who cook in the open air. The part of the individual risk (RA) that can be attributed to the exclusive connection to the type of room constitutes 0.51 for bronchial asthma, 0.47 for bronchitis and pneumonias, 0.46 for strokes, 0.42 for ischemic heart disease and 0.40 for obstructive bronchitis.

People, who cooked the meals on peasant stoves, present a relatively moderate risk (RR) and risk 42.3 times more in comparison to people who prepared food on cookers. If we select the cases when the peasant stove was placed in the living room, the level of the relative risk rises $2.2 \leq RR \leq 2.4$, $p < 0.001$ and is 29.4 times higher than when preparing food on cookers. The relative risk of the contraction of diseases increases in relation to the quality of the fuel used: biomass ($3.3 \leq RR \leq 4.5$, $p < 0.05$), agricultural waste ($3.5 \leq RR \leq 3.6$, $p < 0.01$), hard coal ($3.4 \leq RR \leq 3.5$, $p < 0.01$), in contrast to other types of fuel. In cases when people were smoking in the house, the risk rose ($3.4 \leq RR \leq 5.2$, $p < 0.001$).

The absolute risk of suffering from all nosologic forms is much higher in the case of preparing food on peasant stoves (for bronchial asthma – 0.68, for strokes and obstructive bronchitis – 0.64, for the ischemic heart disease – 0.56, for pneumonias – 0.55, for bronchitis – 0.48).

The respondents who prepared dishes on peasant stoves and used agricultural waste present a lower risk ($RR=1.3$, $p < 0.001$) and can develop bronchitis 18.3 times more often than those who use other types of fuel, those who use biomass ($RR=1.2$) risk 24.4 times more ($p < 0.01$), when using brushwood ($RR=1.4$, $p < 0.05$), individuals risk 10.7 times more ($p < 0.01$), when using wood ($RR=1.2$, $p < 0.001$), they risk 7.5 times more ($p < 0.001$), when using hard coal ($RR=1.3$, $p < 0.05$) – 18.5 times more ($p < 0.01$), when using processed coal ($RR=1.2$, $p < 0.001$) they risk 12.3 times more ($p < 0.01$), in comparison to other types of fuel.

Moreover, the respondents who used agricultural waste in the process of cooking, presented a moderate risk ($RR=2.2$, $p < 0.001$) and risk 25.5 times more to develop obstructive bronchitis, people who use biomass ($RR=1.5$, $p < 0.01$) risk 27.8 times more ($p < 0.01$), and those who use brushwood ($RR=1.4$, $p < 0.01$) risk 9.4 times more ($p < 0.05$), when using wood ($RR=1.5$, $p < 0.001$), they risk 8.5 times more ($p < 0.01$), when using hard coal ($RR=1.7$, $p < 0.01$) they risk 15.1 times more ($p < 0.01$), when using processed coal ($RR=1.5$, $p < 0.01$) they risk 14.2 times more ($p < 0.01$), in comparison to other types of fuel.

Thus, people who used agricultural waste are subjected to moderate risk ($RR=1.9$, $p < 0.01$) to develop pneumonias, 21.3 times more often ($p < 0.001$) than those who use other types of fuel, if biomass is used ($RR=1.7$, $p < 0.001$), people are subjected to a risk 24.4 times higher ($p < 0.01$), if using brushwood ($RR=1.4$, $p < 0.001$) people are subjected to a risk 10.7 times higher ($p < 0.01$), if using wood ($RR=1.5$, $p < 0.001$), people risk 7.5 times more ($p < 0.001$), hard coal – ($RR=1.9$, $p < 0.05$) people risk 18.5 times more ($p < 0.01$), when using processed coal – ($RR=1.8$, $p < 0.01$) people risk 12.3 times more ($p < 0.01$), in comparison to other types of fuel.

In the case when people used agricultural waste in the process of cooking on the peasant stove, they are subjected to very high risk ($RR=2.3$, $p < 0.001$), thus risking to develop bronchial asthma 36.6 times more often than ($p < 0.001$), people who use biomass ($RR=2.1$, $p < 0.01$) risk 28.7 times more ($p < 0.01$), and those who use brushwood ($RR=1.9$, $p < 0.01$) risk 22.3 times more ($p < 0.01$), when using wood ($RR=1.6$, $p < 0.001$), they risk 16.4 times more ($p < 0.001$), when using hard coal ($RR=2.4$, $p < 0.001$) they risk 26.3 times more ($p < 0.01$), when using processed coal ($RR=1.9$, $p < 0.05$) they risk 21.6 times more ($p < 0.01$), in comparison to other types of fuel.

A relatively moderate risk ($RR=2.1$, $p < 0.001$) has been detected for the development of strokes and people who used agricultural waste may have a stroke 33.1 times more often ($p < 0.001$), if biomass is used ($RR=2.1$ $p < 0.05$) people are subjected to a risk 20.7 times higher ($p < 0.01$), if using brushwood ($RR=1.7$, $p < 0.001$) people are subjected to a risk 8.3 times higher ($p < 0.01$), if using wood ($RR=1.8$, $p < 0.001$) people risk 8.1 times more ($p < 0.001$), hard coal – ($RR=2.2$, $p < 0.001$) people risk 14.6 times more ($p < 0.01$), when using processed coal ($RR=1.9$, $p < 0.001$) people risk 11.8 times more ($p < 0.01$), in comparison to other types of fuel. The attributable risk varies between 0.50 and 0.68 for different kinds of fuel used in the case when people who suffer from obstructive bronchitis and strokes: 0.46-0.54 – in the case of people with bronchial asthma, pneumonias and ischemic heart diseases and 0.44 at people with bronchitis. The usage of less harmful fuel could prevent from 54.3% (bronchitis) to 82.5% (asthma) of illnesses.

The lack of ventilation in the kitchens constitutes a lower risk ($1.4 \leq RR \leq 1.6$, $p < 0.01$), in the living room – a very high risk ($2.9 \leq RR \leq 3.7$, $p < 0.001$), in the summer kitchens – a reduced risk ($1.3 \leq RR \leq 1.5$, $p < 0.05$), and if applying ventilation measures, there can be prevented from 32.3% to 41% of specific diseases. The presence and the functioning of the ventilation systems in the kitchens, the presence and the possibility to open the windows, the presence of the ventilation duct in the living room and summer kitchens represent a major factor of protection; in the kitchens – constitute a major factor of protection ($R=0.2$, $p < 0.05$), the living room ($R=0.3$, $p < 0.01$), and in the summer kitchens ($R=0.5$, $p < 0.001$) – factor of moderate protection.

The part of the absolute risk which is directly connected to the lack of an adequate ventilation system constitutes 0.88 for bronchial asthma, 0.64 for the ischemic heart diseases, 0.61 for obstructive bronchitis, 0.54 for strokes and pneumonias, 0.50 for bronchitis. In many spaces where food is prepared the conditions are unfavorable due to the lack of windows or their not being functional. This situation presents an absolute risk of 0.55 for obstructive bronchitis, 0.53 for strokes, 0.46 for the ischemic heart diseases, 0.41 for bronchial asthma, 0.40 for pneumonias, and 0.37 for bronchitis.

Calculating the risk in relation to nosology, it has been established that people who prepared food in the living room on the peasant stove, using biomass, agricultural

waste and brushwood as fuel, the ventilation being insufficient and the respondents being smokers, all of this presents a very high risk of developing bronchial asthma (R=7.9, $p<0.001$), obstructive bronchitis (R=6.5, $p<0.01$), pneumonias (R=7.1, $p<0.001$), strokes (R=6.6, $p<0.001$), the ischemic heart diseases (R=6.4, $p<0.05$) and moderate risk present to develop bronchitis (R=1.9, $p<0.05$).

People who cook dishes in conditions with high temperature and air humidity are put at risk to develop pneumonias (R=4.2, $p<0.01$), strokes (R=3.7, $p<0.001$), ischemic heart diseases (R=3.5, $p<0.001$), risk moderately to develop obstructive bronchitis (R=1.8, $p<0.001$), bronchial asthma (R=1.8, $p<0.001$) and present very high risk to develop bronchitis (R=1.4 $p<0.001$). These people risk 38.6-67.4 times more than people who cook at lower values of physical factors. The absolute risk based on the modification (surpassing the norms) of the physical factors from the cooking spaces constitutes 0.53 for obstructive bronchitis, 0.51 for bronchial asthma, 0.48 for pneumonias, 0.47 for bronchitis, 0.46 for ischemic heart diseases and 0.40 for strokes.

The concentration which exceeds the norm of carbon dioxide presents a very high risk in developing bronchial asthma (R=8.5, $p<0.05$), ischemic heart diseases (R=6.8, $p<0.01$), obstructive bronchitis (R=5.5, $p<0.001$) and reduced risk to develop bronchitis (R=1.4, $p<0.01$), pneumonias (R=1.4, $p<0.001$), strokes (R=1.2, $p<0.001$). People subjected to excess carbon dioxide risk 46.8-63.4 times more than the ones who cook in conditions with normal concentrations of carbon dioxide. The undesirable effect (RA) upon the population exposed to the higher concentration of carbon dioxide is 0.47 for bronchial asthma and obstructive bronchitis, 0.43 for the ischemic heart diseases, 0.41 for pneumonias and bronchitis and 0.36 for strokes.

In cases when carbon monoxide exceeds the norm, the population risks very much to develop bronchial asthma (R=7.1, $p<0.001$) and obstructive bronchitis (R=6.3, $p<0.01$), risks moderately to develop strokes (R=2.4, $p<0.001$), ischemic heart diseases (R=2.4, $p<0.01$) and present a low risk to develop bronchitis (R=1.5, $p<0.01$) and pneumonias (R=1.2, $p<0.05$).

People who prepare food in conditions with increased concentrations of carbon monoxide risk 67.4-72.1, four times more than those who cook in conditions with normal carbon monoxide concentrations. The absolute risk of the appearance of nosologic forms specified by the respondents who cook in rooms with carbon monoxide concentration that exceeds the norm is also different and constitutes 0.67 for the ischemic heart diseases, 0.55 for bronchial asthma, 0.53 for strokes, 0.50 for obstructive bronchitis, 0.46 for pneumonias and 0.45 for bronchitis.

Reducing the duration and the frequency of food preparing, excluding the effect of the physical and chemical factors in the rooms attributed to cooking meals, there can be prevented 56.0% of cases of obstructive bronchitis, 54.3% of bronchial asthma, 51.3% of ischemic heart diseases,

45.8% of the cases of pneumonias, 44.6% of bronchitis, 29.4% of cases of strokes.

Conclusions

The results of this research may help the factors of decision to become aware of the need of educational programs intended to ensure the inhabitants with information about the sources of pollution of the indoor air and the risks they are subjected to. It is necessary to focus our effects upon the communication strategy to motivate the direct personal perception or to become aware of the environmental problems, such as air pollution. Such an approach enhances the understanding of the importance of the measures taken by the environmental policies, which make these measures easier to be received by the inhabitants, as well as to improve the personal feedback in reducing the impact of pollutants.

Without a substantial change in the policies, the total number of people who depend on solid fuel will mostly remain unchanged. The use of the pollutant fuel represents a major burden for steady development. There is also acute need of additional research in the public perception area, in order to help to understand the factor which shapes human perception. The project was done during summertime. It would be necessary to carry out a similar project with measurements during winter.

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REVIEW ARTICLES

The essence of the cell therapy method in chronic ischemia of the lower limb

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Abstract

Background: The results of the of cell therapy use in experimental models of ischemia of the lower limbs, in the majority of cases performed on mice and rats, and also on bigger animals demonstrated the possibilities and effectiveness of cell therapy in restoring blood flow to ischemic regions of the member. The third important property of stem cells is that they can be differentiated into diverse specialized cell types, such as muscle cells, endothelial, nervous, etc. Stem cells are the basic unit of the body, from them are formed 240 types of specialized cells and tissues of the body. These cells are involved in the revascularization and increase the secretion of numerous pro-angiogenic factors (VEGF, bFGF, PlGF-1 and MCP-1). All preclinical studies demonstrate a local neovascularization, with a functional effect, particularly in the form of increased blood flow and the number of newly formed vessels. Aspects and perspectives of cell therapy in the treatment of chronic peripheral angiopathy, especially on lower limbs are considered in this work.

Conclusions: Angiogenic cell therapy today provides a therapeutic method and also an innovation that represents a breakthrough concept in treatment of ischemic diseases, especially of the legs and heart, the improvement of prognosis of these patients. This method comes to substitute the conventional methods of treatment of these diseases, which until now claim to be less effective or even ineffective, and simultaneously takes an important step in the progress of therapy, which further enriches the arsenal of methods of medical treatment. Cell therapy is one of the most perspective methods of treatment at the current stage, with the use of mature autologous mesenchymal cells, in order to increase the number of capillaries (angiogenesis), and larger caliber vessels (vasculogenesis).

Key words: ischemia, stem cells, angiogenesis, regeneration.

Introduction

The essence of cell therapy method consists in active substitution and stimulating effect on the functional deficient cells and tissues in some organ and separate systems, stimulating reparative and metabolic processes, immunocorrection, immunostimulation and stimulation of angiogenesis using stem cells [16]. At the base of developed technology of drugs manufacturing from stem cells for cryopreservation and treatment is the provision on necessity of preservation by biological objects of the viability, meaning their possibilities of functioning after defrosting and introduction into the body, which ensures high clinical outcomes [1]. The advantage of cell therapy is receiving by the patient a number of biologically active and balanced compounds of natural origin, which may influence the variety of whole body metabolism, and also stem cells are able to perform replacing functions [2].

Angiogenesis represents a multistage process involving changes at the level of the extracellular matrix, proliferation of endothelial cells, migration and differentiation of them into new capillaries. Studies on tumor biology have led to the discovery of complex interactions (autocrine and paracrine) between tumor cells, stromal cells and endothelial cells being under the influence of the extracellular matrix composition. In the last years it has become increasingly evident that the formation of new vessels is the result of the interaction of different proangiogenic and antiangiogenic molecules, represented by growth factors

and some components of the extracellular matrix. The study of angiogenesis and its role in tumor biology led to a vast theoretical baggage as well as to some results in the treatment of cancer by clinical studies of some drugs with antiangiogenic effect [4, 1, 2]. Microvasculature is a dynamic system that plays an important role in various physiological and pathological processes evolving from a sleeping to an active status. New vessels can arise after the process of angiogenesis, vascular remodeling and recruiting precursor of endothelial cells from bone marrow and blood vessels [5, 3]. Angiogenesis is a process that also depends on the cooperation and the interaction between different cell types, growth factors and various extracellular matrix components.

Stem cells are defined by three main features. First of all, they are not specialized cells (unlike the cells, from that are formed muscles, brain, blood) Secondly, the stem cells are able to divide a long period of time, and, as a result of the division, it forms two identical cells [17]. The third important property of stem cells is that they can differentiate into diverse specialized cell types, such as muscle cells, endothelial, nervous, etc. Stem cells are the basic unit of the body, from them are formed 240 types of specialized cells and tissues of the body. A large number of stem cells are contained in the umbilical cord blood, in the human embryo, in the placental complex, in the bone marrow of an adult. The main characteristic of them is the ability to self-sustaining [6, 13]. Cell therapy or regenerative one

allowed to obtain clinical results of new quality in various areas of medicine: gerontology, oncology, gynecology, hematology, immunology, endocrinology, cardiology, obstetrics, psychiatry, neurology and neurosurgery, surgery, traumatology.

Sources of obtaining progenitor cells:

- Bone Marrow stromal cells
- Multipotent adult progenitor cells – MAPC
- Human umbilical cord stem cells
- Hematopoietic stem cells
- Neural stem cells
- Embryonic stem cells
- Nuclear transplantation of embryonic stem cells.

After the origin progenitor cells (stem) are divided into two groups:

1. Embryonic cells, fetal
2. Mature mesenchymal cells.

The most frequent source of embryonic stem cells is blastocyst. These cells can give birth to any cell lines of the body in respective conditions of the growth and differentiation [18].

Disadvantages: may cause appearance of tumors by embryonic genesis (teratomas).

Advantages:

1. Present pronounced multipotention.
2. Source of them may serve placenta and umbilical cord which may be isolated from these cells then cryopreserved and kept for the duration of the individual's life
3. Possess a weak antigenic load.

Albeit umbilical cord blood contains a smaller number of cells in comparison with bone marrow, the quality of the graft is superior toward the bone marrow.

Mature mesenchymal progenitor cells:

- Can be isolated from bone marrow of the patient.
- They are a subpopulation of cells from bone marrow.
- Are distinguished in a long row of cells in vitro and in vivo.
- Do not form teratoma.

Disadvantages: Do not possess multipotention of embryonic cells. In some cases can exist contraindications for harvesting these cells such as chronic alcoholism, smoking, treatment with cytostatics and glucocorticosteroids, intolerance to proteins of animal origin and specific cytokines, patients with chronic kidney disease and diabetes. With age the reserves of these cells decrease [7, 14, 15].

The effects of progenitor cells

- Normalize and stimulate the metabolism;
- Increase immune and neuroendocrine activity;
- Possess expressed antitumor effect;
- Slow down premature aging, rejuvenating the organism;
- Have a marked therapeutic effect for a wide range of pathologies;
- Anti-inflammatory;
- Immunomodulation;

- Angiogenic effect, improve vascularization;
- Stimulate cell regeneration;
- Replace the affected tissue.

The role of endothelial progenitor cells (EPC) in the cell therapy

In 1997, T. Asahara [21] found in peripheral blood endothelial progenitor cells (EPC) which, after isolating and multiplying *ex vivo* are able to stimulate post-ischemic angiogenesis [3]. EPC Series originating from marrow and expressing the antigen CD34 + plays an important role during postnatal vasculogenesis in physiological and pathological conditions. HSC and EPC derived from bone marrow or peripheral blood, help the process of post-ischemic neovascularization in adults [4]. It is revealed that the affected tissue by ischemia triggers an increase in the concentration of systemic pro-angiogenic growth factors (VEGF-A, PlGF-1, SDF-1, EPO), thus induce progenitor cell mobilization from bone marrow into peripheral circulation. These stem cells, reaching the ischemic territory (“homing”) adhere to the endothelium, where they are incorporated into new groups which then differentiate into endothelial cells. Moreover, progenitor cells produce factors capable of stimulating angiogenesis and vascular remodeling. However, the molecular mechanisms of these cells stage are largely unknown. Indeed, various phenomena, such as the mechanisms for targeting (“homing”) to the site of neovascularization, the nature of these signals, the involvement of growth factors, the role of adhesion molecules are studied further. The discovery of medullar origin EPC in the peripheral blood of adults allowed the development of strategies of proangiogenic cell therapy in ischemic diseases. The administration of EPC or bone marrow cells, intravenously or directly to the ischemic territory, contributes to the stimulation of neovascularization in various animal models [5]. Similarly, injection of CD34 + cells significantly improves post-ischemic angiogenesis in a model of heart failure, and lower limb ischemia [5, 9]. Stem cell therapy may also be based on the administration of bone marrow mononuclear cells. Autologous transplantation of bone marrow-derived mononuclear cells stimulates neovascularization in the leg or in the infarcted heart in various species of animals [8, 19]. Finally, the administration of mesenchymal stem cells improves the process of post-ischemic neovascularization. These cells are involved in the revascularization and increase the secretion of pro-angiogenic numerous factors (VEGF, bFGF, PlGF-1 and MCP-1) [10]. All preclinical studies demonstrate a local neovascularization, with a functional effect, particularly in the form of increased blood flow and the number of newly formed vessels. The results of the use of cell therapy in experimental models by ischemia of the lower limbs, in most conducted in mice and rats, and also on bigger animals have demonstrated the effectiveness of cell therapy possibilities and restoration of blood flow in ischemic regions of the member [20]. It demonstrated increase of the number of circulating endothelial progenitor cells (EPCs)

in response to experimental ischemia [11], and subsequent experimental demonstration of ingress into capillaries and arterioles of these cells in the affected tissue.

The antiangiogenic system is a group of active molecules aimed at inhibiting the process of angiogenesis. In normal tissue there is a well-established balance between angiogenesis and anti-angiogenesis process that maintains constant the number of blood vessels depending on the functional needs of the tissue [12]. As antiangiogenic factors may serve some drugs: carboxyamidotriazole, itraconazole, chemotherapy preparations used to treat malignant tumors with the purpose to inhibit, or arrest tumor angiogenesis [16].

Conclusions

Angiogenic cell therapy today provides a therapeutic method and also an innovation that represents a breakthrough concept in treatment of ischemic diseases, especially of the legs and heart, the improvement of prognosis of these patients. This method comes to substitute the conventional methods of treatment of these diseases, which until now claim to be less effective or even ineffective, and simultaneously takes an important step in the progress of therapy, which further enriches the arsenal of methods of medical treatment.

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Cold moxibustion

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Abstract

Background: Cold moxibustion is a treatment method of Traditional Chinese Medicine, having a secular history and until now it is widely used in the Chinese People's Republic. Treatment consists in applying for a short time herbal powder on the acupuncture points. Cold moxibustion has good effect in the treatment and prophylaxis of respiratory, digestive, osteoarticular, gynecological and nervous system diseases. Thus, ancient Chinese doctors believed that external application of herbs powder can treat internal organs. There are over 200 prescriptions of cold moxibustion.

Conclusions: Clinical research demonstrates that cold moxibustion is an effective method, very simple to apply and inexpensive. Therefore, it would be beneficial to be included in the complex treatment of the respiratory, digestive, osteoarticular, gynecological and nervous system diseases. Cold moxibustion is an ancient method, easy to apply, inexpensive and can be widely used. This method is an ecological one and does not have side effects, is easily bearable by patients. Numerous clinical experiences made in the Chinese People's Republic demonstrate high efficiency of this method.

Key words: cold moxibustion, traditional Chinese medicine.

Introduction

Cold moxibustion is a treatment method of Traditional Chinese Medicine. It consists in applying for a certain period of time, a mix of Chinese herbs powder in areas of acupuncture points. Because sometimes there can occur hyperemia and blisters as in the moxa therapy, the method was called Cold moxibustion. Traditional method is used in summer heat, known in China as "San fu tie", period, but can be used at any time of the year. In translation "San" means three signifying the start, middle and end of hot summer, "fu" indicates the heat, "tian" means a day. "San fu tie" period has three sub-periods, with total duration of 30-40 days and begins after the summer solstice. This period is calculated each year by the lunar calendar using a complicated formula, so the period will be different every year. Thus in 2017 "San futien" will debut with "ChuFu", sub-period which will last 10 days (12.07-21.07), then follows "Zhongfu" sub-period lasting 20 days (22.07-10.08) and the last is "MoFu", sub-period which lasts 10 days (11.08-20.08).

History

For the first time this method has been mentioned in the work "Requirements for 52 diseases", where the application of white mustard seed *Semen Sinapsis* on Baihui point (GV20) for snakebites is recommended. In another ancient work "Shengnong's Classic of Materia Medica" it is mentioned that the application of *Lytta vesicatoria* is effective in skin ulcers and gangrene. A famous ancient physician Sun Si Miao in his work "Thousand Golden Recipes" wrote that *Herba Ecliptae* powder applied on Jianshi point (PC5) heals you. In the ancient famous work "Taiping Holy Preions for Universal Relief" it is mentioned that for the treatment of back and legs, *Radix Aconite* is applied on

umbilical region. Thus ancient Chinese doctors believed that external application of herbs powder can treat internal organs. There are over 200 prescriptions of cold moxibustion.

Mechanism of action

Cold moxibustion treatment is based on the concept called: "winter diseases treated in summer". In summer Yang energy is on body surface, pores are open and this facilitates faster absorption of herbs powders and stimulates the points. Through the phytotherapeutic action of the plant and acupoint stimulation can be obtained strength Yang energy, which disperses internal cold, invigorates kidneys and spleen, meridians drainage, regulating the flow of Qi and blood, thus the immune system strengthens, receiving a treatment and prophylactic effect.

1. Local Action. Cold moxibustion has a vasodilatation effect, so it improves microcirculation and tissue adjacent power, also it decreases edema.

2. Action on the nervous system. By stimulating peripheral nerve fibers or their inhibition the regulation of internal organs function occurs.

3. Action on the immune system. By regulating cellular and humoral immunity the treatment effect is obtained.

Modern research shows that cold moxibustion acting through the sympathetic and parasympathetic system can change the contraction and relaxation of vascular smooth muscle and bronchial smooth muscle and secretory gland, reducing state of hypersensitivity of the airways and improve body non-specific immune function [1]. Cold moxibustion increases macrophage phagocytosis, increases the rate of transformation of lymphocytes and plasma cortisol, reducing the number of eosinophils in the blood [2]. By stimulating the acupuncture points and through the

absorption, metabolism of plants occurs nervous, endocrine and immune system regulation, significantly increases biological potency in vivo, generating impact on the lungs physical, chemical receptors and decrease IgE serum levels, by stimulating cerebral cortex occurs autonomic regulation manifested by improving the body's immune response and increasing resistance to disease [3].

Contemporary research

Action on leukocytes

Sun Di Li [4] demonstrated that cold moxibustion with different concentration of *Lytle vesicatoria* applied on mice with chemotherapy on points Dazhui (GV14), Shenshu (BL23), Zusanli (ST36), produces bone marrow hyperplasia and rapid growth of leukocytes numbers in comparison to the control group. Also Sun Di Li [5] clinical research demonstrated that cold moxibustion with *Lytle vesicatoria* tincture can stimulate macrophage colony growth factor (GM-CSF) in the peritoneum, thus stimulating the growth and differentiation of hematopoietic stem cells and increasing the leukocytes number in peripheral blood. Obtained laboratory data can be used for developing combined treatment methods of leukemia and tumors. The author has used cold moxibustion for treatment of glioma with satisfactory results.

Action on T lymphocyte

Zheng Qian [6] investigated Cold Moxibustion action on the CD4 / CD8 report on rats with asthma. The study found the reduction of CD4 / CD8 ratio in the material taken from the trachea and spleen of rats with allergic asthma. Yong Rongxue [7] used cold moxibustion on patients with chemotherapy and there has been found decreased white blood cell counts and increased lymphocyte proliferation.

Clinical use

Respiratory disease

Zhang Qingzhen and others [8] applied cold moxibustion method to the 1500 asthmatic patients, treatment efficiency is 99.07%. The author used the following plants: *Semen sinapis*, *Euphorbia Kansai T. N. Liou ex T. P. Wang*, *Asarum sieboldii Miq*, *Ephedra sinica Stapf*, *Corydalis turtschaninovii Bess. f. yahusuo Y. H. Chou et C. C. Hsu* etc. The used points: Feishu (BL13), Xinshu (BL15), Pishu (BL20), Shenshu (BL23), Wei Zhong (BL40), Dingchuangxue (EX-B1). Liu Guocheng, Han Genyan [9] used Cold Moxibustion method to treat 145 patients with asthma, treatment efficiency – 86.9%. The authors used the plants: *Semen sinapis*30g, *Euphorbia Kansai TN Liou Ho SB* 15g, *Asarum sieboldii Miq*-15g, *Corydalis turtschaninovii Bess. f. yahusuo H. Y. Chou et C. C. Hsu*15g, *Magnolia biondii Pamp*15g. Plants powder mix with ginger juice are applied on points Feishu (BL13), Fenmen (BL12), Gaoyushu (UB26) for about 2 hours. Du Xu [10] used powder *Semen sinapis*, *Ephedra sinica Stapf*, *Asarum sieboldii Miq*, *TN Liou ex Euphorbia Kansai Ho SB*, *Rizoma Coryda-*

lis, *Rizoma Peniliae* for the treatment of 30 patients with chronic bronchitis, treatment efficacy was 93.3%.

Nervous system diseases, Rheumatism and other osteoarticular diseases

Cold moxibustion is widely used to treat rheumatism and arthritis because by stimulating specific points, meridians and collaterals can be drained, regulating the flow of Qi and blood; also analgesic effect can be achieved [11].

Zhao Xinkui [12] investigated the action of Cold moxibustion on values of IgA, IgG, IgM, CRP, ESR in patients with rheumatism. After the treatment an improving of the indices listed was obtained. Treatment efficiency in research group was 92.5%. The scientist selected such plants as: *Asarum sieboldii Miq plants*, *Semen sinapis*, *Herba Spiraniskiae tuberosa*, *Euphorbia Kansai TN Liou Ho SB ex*, *Fructus Evodiae*, *Moschus*, *Flos Dturae*, which were applied on points Dazhi (DU14), Fenglong (ST40), Quchi (LI11), Yinling Quan (SP9) at the beginning, middle and end of the “San futien” period for 4-6 hours.

Zhang Jin [13] used cold moxibustion in 54 cases of scapulohumeral arthritis. Selected plants: *Semen sinapis*, *Asarum sieboldii Miq*, *Radix aconite*, *Ramulus Cinnamomi*, *Cortex Cinnamomi*, *Radix Angelicae Dahuricae*, *Rhizoma Kaempferiae*, mixed with ginger juice were applied on Tai Yuan (LU9) point. As a result of the treatment there were registered: healing – 35 cases, improvement – 14 cases. Total efficiency – 90.17%.

Zhang Feng [14] used a combination of cold moxibustion and acupuncture for the treatment of 24 patients with Facial Neuropathy. Selected plants were: *Fructus Crotonis*, *Mylabris*, *Curcuma Longa*. In 20 cases very good results were obtained, in 4 cases – good result, treatment total efficiency – 100%. As a result of local treatment adverse effects such as burns or scars have not been found.

Xie Honglian [15] and others used applications of *Semen sinapis*, *Asarum sieboldii Miq*, *Euphorbia Kansai T. N. S. Liou ex B. Ho*, *Rizoma Corydalis* for treatment of 60 cases of Cervical Spondilopati. The applications were used in the first 10 days and lasted 10 days of summer hot period on points Dazhui (Du14), Tianzhong (SI11), Jianyu (LI15), Quchi (LI11), Waiguan (SJ5), Hegu (LI4). Total efficiency – 91.67%.

Diseases of the digestive system

Cold moxibustion is used in the treatment of gastritis caused by *Helicobacter pylori*, stomach pain, irritable bowel syndrome, ulcerative colitis and gastric ptosis. Yuan Jianrong [16] and others used the Cold moxibustion method to treat chronic stomach pain. There were selected the following plants: *Semen sinapis* – 40g, *Asarum sieboldii Miq* – 40g, *Euphorbia Kansai TN Liou Ho SB ex* – 10g, *Rizoma Corydalis* – 10g. In the early hot period the selected points were Guanyuan (RN4), Zhongwan (RN12), Tianshu (ST25), Zusanli (ST36), in the mid hot period the selected points were Xiawan (RN10), Shangwan (RN13),

WeiShu (BL21), Shangjuxu (ST37) during the hot terminal period there were selected Neiguan (FP6), Gongsun (SP4), Pishu (BL20), and in the last two days of the terminal period points of hot early or middle period were again selected. Treatment efficiency – 87.95%.

HeYueshuo [17] used the powder of *Fructus Evodiae*, *Semen Myristicae*, *Semen sinapis*, *Rhizoma Corydalis*, *Cortex Cinnamomi* mixed with ginger juice applied on Tianshu (ST25), Guanyuan (RN4), Zusanli (ST36), Pishu (BL20), Shengque (RN8) points to treat diarrhea. Total efficiency was 97.8%.

Shi Xiaowen [18] used “Baijiezan” powder made from *Semen sinapis*, *Asarum sieboldii* Miq, *Euphorbia kansui* T. N. Liou ex S. B. Ho, *Rizoma Corydalis*, applied on the points Guanyuan (RN4), Tianshu (ST25), Qihai (RN6) to treat colitis. Treatment efficacy was 93.7%.

Gynecological diseases

Yang Haizheng [19] for the treatment of 38 patients with Dysmenorrhea, combined administration of “Zi Ni Nuǎn Qu Yū Tang” decoct with cold moxibustion. Used plants: *Semen sinapis*, *Asarum sieboldii* Miq, *Herba Ephedrae* and *Coridalis yanhusuo* applied on Guan Yuan (RN4), Qihai (RN6), Sanyinjiao (SP6), Ciliao (BL32) points. The efficiency was 94.7%.

Zhang Yuayuan [20] used external applications of garlic and Mirabilite to treat 115 patients with acute mastitis. Healing – 89cases, improvement – 26 cases. Total efficiency was 100%.

Pediatric diseases

Wei Zhizhong and others [21] used cold moxibustion for the treatment of 30 children with enuresis. Selected plants were: *Rhus chinensis* Mill, *Alpinia oxyphylla* Miq., *Tenodera sinensis*, *Rose laevigata*, *Polygala tenuifolia* Willd, applied on Yongquan points (KI1), Shenque (CV8). As a result of the treatment healing was found in 12 cases, improvement – 18 cases, total efficiency – 100%.

Mo Shan [22] and others used cold moxibustion for the treatment of 120 children with Tourette syndrome. Used plants were: *Semen sinapis* and *Asarum sieboldii* Miq. Cold moxibustion was applied on Feishu (BL13), Pishu (BL20), Shenshu (BL23) and Tiantu (LU3) points. The treatment was used for children older than 5 years old, in hot initial and hot final period. After 1 month of treatment the frequency of tics was significantly decreased ($p < 0.01$).

Conclusions

Cold moxibustion is an ancient method, easy to apply, inexpensive widely used for the treatment and prophylaxis of respiratory, digestive, nervous, gynecological and rheu-

matological diseases. This method is ecological one and does not have side effects, is easily bearable by patients. Numerous clinical experiences made in the Chinese People's Republic demonstrate high efficiency of this method.

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6. **Referințele**, în conformitate cu cerințele Comitetului Internațional al Editorilor Revistelor Biomedicale (www.icmje.org, capitolul IV.A.9), se expun în ordinea apariției în text. În lista referințelor titlul articolului, se traduce în limba engleză, poziționându-se în paranteze pătrate. Referințele bibliografice prezentate în grafie chirilică sunt transliterate în grafie latină, utilizând următoarele semne grafice: A-A, B-B, B-V, G-G, D-D, E-E, E-E, Ж-ЖН, З-З, И-И, Й-Й, К-К, Л-Л, М-М, Н-Н, О-О, П-П, Р-Р, С-С, Т-Т, У-У, Ф-Ф, Х-ХН, Ц-ЦС, Ч-ЧН, Ш-ШН, Щ-ЩН, Ы-Ы, Э-Э, Ю-Ю, Я-Я; Ъ și Ы se omit. Imediat după transliterare, în paranteze pătrate, se prezintă traducerea titlului articolului în limba engleză. De exemplu: Ivanov IV, Sidorov VM, Kozlov NE. Transplantatsiya organov i tkaney [Transplantation of organs and tissues]. Vestnik Khirurgii [Messenger of Surgery]. 2010; 26(6):45-49.

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