ISSN 2537-6373 (Print)
ISSN 2537-6381 (Online)

The Moldovan Medical Journal

The Publication of the Scientific Medical Association of Moldova

Frequency – 4 per year

Vol. 61, No 1
February 2018

Welcome to the Moldovan Medical Journal!

The Moldovan Medical Journal is an international scientific double-blind peer reviewed periodical edition, 6 per year, of the Scientific Medical Association of the Republic of Moldova designed for specialists in the areas of medicine, dentistry, pharmacy, social medicine and public health. From its debut 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 new and more effective ways of solving numerous medicine problems. We hope that those who want to make their contribution to the science of medicine will find our journal helpful and encouraging.

Boris Topor, MD, PhD, Professor
Editor-in-Chief

Editorial Board

Advisory Board

Emeritus Members of the Advisory Board

CONTENTS

RESEARCH STUDIES

REVIEW ARTICLES

BOOK REVIEW

Editorial Staff

Martinenko Liudmila, English Corrector, Telephone: +37322205209
Guzun Marina, Editorial Secretary, Telephone: +37332205877

Printing House "Tipografia Sirius"
2, A. Lapusneanu str., Chisinau, MD-2004
the Republic of Moldova
www. sirius.md

RESEARCH STUDIES

DOI: 10.5281/zenodo.1186164
UDC: 613.292:615.1

Pharmacists' role in managing food supplements

Dogotari Liliana, PharmD, PhD, Associate Professor, ***Adauji Stela,** PharmD, PhD, Associate Professor

Vasile Procopisin Department of Social Pharmacy, Faculty of Pharmacy
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
*Corresponding author: stela.adauji@usmf.md. Received December 15, 2017; accepted February 05, 2018

Abstract

Background: Every year, the value of the world market of Food Supplements (FS) increases considerably, especially following the accepting the role of nutrition in maintaining health. The share of official importers of medicinal products on FS market is not significant and the procedure for placing food supplements on the market is not sufficiently regulated. All these conditions create major health risks for FS consumers.
Material and methods: In order to study the complexity of the field of food supplements and to highlight the existing problems in this field at the national level, and also to determine the pharmacist's role in releasing food supplements and counseling patients, was used comparative method for registering the dietary supplements in Romania and the Republic of Moldova.
Results: The complex assessment of food supplements in the Republic of Moldova and Romania and elucidation of the pharmacist's role in dispensing of food supplements were carried out in order to prevent the abuse and non-rational use of the FS. For this reason it was carried out a comparative analysis of the legal framework for FS in both mentioned countries and the study of the national food supplements market was performed according to the criteria of the producers and importers that are present on the market.
Conclusions: Legislation on food supplements in the Republic of Moldova is incomplete and unsatisfactory formulated. The value of the food supplements market is constantly increasing making this area attractive. The prevalence of economic interests over the medical interests may have adverse consequences for the health of the population. The pharmacist has the primary role both in implementing the necessary legislative changes and in counseling patients to prevent the non-conforming use of food supplements.

Key words: food supplement, legislation, food supplements market, risk, population health.

Introduction

As early as in 90s, the food supplements market (FS) was almost non-existent in the Republic of Moldova and as well, in its neighboring countries, which is a total difference from the current situation. Excessive consumption of food supplements (FS) is becoming more and more current issue, taking into consideration the concern of modern people about healthy living and eating, as the environment becomes more unfriendly and the life more stressful.

Along with the benefits that population has through the technology in the most areas of life, this is also a prerequisite for the environmental degradation of the environment.

The ecological issue has been worldwide discussed and considered one of the main global problems faced by people. There are a lot of studies carried out all around the world, demonstrating the damage caused by the ecological status of the environment and its effects on the quality of life. According to WHO data till 2050 around 25% of the European population will be over 65, a large number of countries face demographic challenges, the aging of the population, etc. Under these conditions, an increased attention is paid to healthy lifestyle: reducing stress, physical activity and, last but not least, an appropriate nutrition regime. Due to this reason, a favorable condition for the development of the FS market has been created [1]. FS have begun to extend their coverage area in the EU due to the widespread recognition of the role of nutrition in maintaining health. FS consumption, as well as consumer confidence in their effects increases each year, constituting an easy solution to the problem of maintaining health in the accelerated pace of contemporary life.

The global market value of the FS has exceeded $ 80 billion USD, on the background of modern people concern for a proper health status and quality of the life [2].

Based on the Directive 2002/46 / EC of the European Parliament and of the Council of 10th of June 2002 on the harmonization of the legislation of the Member States relating to food supplements, "food supplements" means food stuffs intended to supplement the nutrition and which are concentrated nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed via different forms of issue, such as capsules, pills, tablets, powders, liquid in vials, other similar forms of liquids and powders intended to be taken in small measured unit quantities. In the "Dietary Supplement Health and Education Act of 1994" it is foreseen that FS are food products and not medication.

The phenomenon of the increase of FS consumption is

obvious also in the Republic of Moldova, having, in principle the same premises as in the rest of the countries with a different level of development. However, despite this, regulating the registration and circulation of FS on the Moldovan market, controlling the consumption of the FS, monitoring the adverse reactions and advising the consumer on FS – need to be improved [3, 4, 5]. The incomplete legislation of the Republic of Moldova and the lack of a set of 7 good practices in the management of the circulation of the FS, the advertising that may suggest to the consumer erroneous ideas about the effects of the FS, may consequently lead to the inappropriate and uncontrolled consumption of FS and, as a result, compromising the consumer health [6]. The possibility of purchasing FS without a prescription, not only in pharmacies, but also in other ways (online shops, teleshopping, drugstores, supermarket, natural products' shops, etc.), deprive the consumer of getting any professional advice and the often inconsistent advertisement becomes the sole source of information for the population.

In this context, we intend to evaluate the complexity of factors that have contributed to the current situation in the food supplements market in the Republic of Moldova.

Material and methods

The research was conducted to study the complexity of the field of food supplements and highlight the existing problems in this field at national level, as well as to determine the role of the pharmacist as the main specialist in the field of dispensing food supplements and counseling the population to prevent abuse and non-adequate use of food supplements.

The study, analysis and synthesis of literature data regarding the circulation of food supplements on Romanian and Moldovan markets by using the interpretative and comparative method of the legislation and procedures for registration and placing of food supplements on the market have been carried out with the purpose to propose different ways to improve the regulation and monitoring of FS circulation on the national market.

The comparative method involves comparing the procedures of registering food supplements in Romania and the Republic of Moldova, comparing the content of the food supplements dossiers and assessing the differences in order to determine some possibilities for improving the procedure of registering food supplements.

The interpretative method involves performing statistical surveys of the national food supplements market based on the criteria of FS manufacturers, importers and distributors and interpreting the results obtained.

Results and discussion

According to the legislation of Romania and the Republic of Moldova, food supplements are not included in the category of medicines, therefore the procedure for their registration and placing on the market differs from the one for medicinal products.

In Romania, the procedure for registering food supplements differs depending on their composition. Food supplements (vitamins and minerals) are registered directly in the Ministry of Health, and the FS containing other substances for nutritional and physiological purposes are registered at the Institute of Food Bioresources within the Ministry of Agriculture and Rural Development or at the Regional Centers of Public Health in Cluj-Napoca, Timisoara or Iasi, belonging to the Ministry of Health.

In the Republic of Moldova, the registration and notification procedure is carried out at the National Public Health Center (NPHC), food supplements, containing nutrients are subject to the registration procedure and those containing other substances for nutritional and physical purposes are the subject of notification procedure.

According to the legislation, the food supplement manufacturer or the person responsible for placing the food supplement on the market fulfills a registration form to which the product label is enclosed in the folio and the food supplement dossier is going to be registered.

The content of the file attached to the request for notification is different in both countries (tab. 1). The legislation of the Republic of Moldova on the procedure for registration and notification of FS is incomplete and is incorrectly formulated. General notions are used, without clarifications, which make it possible to avoid the necessary rules. (e.g.: «documents and information ....», «statements ...», «evidences ...», etc.). It is not mentioned the nature of the document by whom they should be issued and confirmed.

Table 1

The comparative content of the FS dossier in Romania and the Republic of Moldova

No	Republic of Moldova	Romania
1.	Information about the applicant's name and his legal address	Application for Product Notification
2.	Identification data of manufacturer and importer (if applicable)	Certificate of registration at the Trade Register, its copy
3.	Place of production, trade name of the product	Product Presentation Format for Import Products - Certificate of Conformity and Country of Origin
4.	Original label design and the one with its translation into the state language	List of product ingredients (quantitative and qualitative)
5.	Documents and information certifying that the components of the product are legally manufactured and marketed in the country of origin	Physico-chemical and microbiological analysis bulletin issued by a laboratory accredited by a third party
6.	Evidence of the components of the product	Product label in Romanian
7.	Declaration/Statement that the product is not registered as a medicine in the country of origin	Packaging certificate, issued in accordance with the legislation in force

In 2013, in order to improve the regulations in the field of food supplements in the Republic of Moldova, a draft Government Decision on the notification of food supplements was developed, representing a transposition of:

- Directive 2002/46 / EC of the European Parliament and of the Council of 10th of June 2002 on the approximation of the laws of the Member States relating to food supplements.
- Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamins and minerals and their forms that can be added to foods, including food supplements.
- Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20th of December 2006 on nutrition and health claims made on foods.
- Commission Regulation (EU) No 432/2012 of 16th of May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children's development and health.

Recent data on national market values of food supplements are not visible in the information flow.

In this paper we conducted the statistical analysis of the market by producers, importers and distributors of FS on the Moldovan market.

As a database served the Register of Food Supplements of the National Centre of Public Health, which includes the following lists:

- The list of registered food supplements – 813 trade names
- The list of food supplements notified – 1696 trade names
- The list of nutritionally and physiologically registered substances – 3 trade names
- The list of food supplements which have been refused for the registration – 1 trade name

The market share of the producers of food supplements registered and notified in the Republic of Moldova is demonstrated in figure 1.

According to the results, almost 30% represents the group of importers holding less than 1% on the national market of the FS. The other 70% constitute 20 importers, from which six are official medicine distributors: Tetis International, Prosanitas Farm – 1%; Esculap-Farm, Farmina SRL – 3%, Dita EstFarm – 4%, IM Becor SRL – 8%. The leader is the "Sibimport" importer with 9% (tab. 2).

Table 2

Share of (%) of FS importers registered in the Republic of Moldova by 01.10.16

No	Importer	Market share, %
1.	Tetis International	1
2.	Prosanitas Farm	1
3.	Vainstein Sanatate	1
4.	SC Farmaco Social	2
5.	Moebius SRL	2
6.	Darurile Vietii	2
7.	Virim Impex	2
8.	Vivasan	2
9.	PVR Stil SRL	3
10.	Farmina SRL	3
11.	Esculap-Farm	3
12.	Forever	3
13.	Maiac-Farm	3
14.	Ocean Resourse	4
15.	Zezifora	4
16.	Dita EstFarm	4
17.	Coral Club	6
18.	Cosmovis	7
19.	ÎM Becor SRL	8
20.	Sibimport	9
21.	Others (importers with quota < 1%)	30

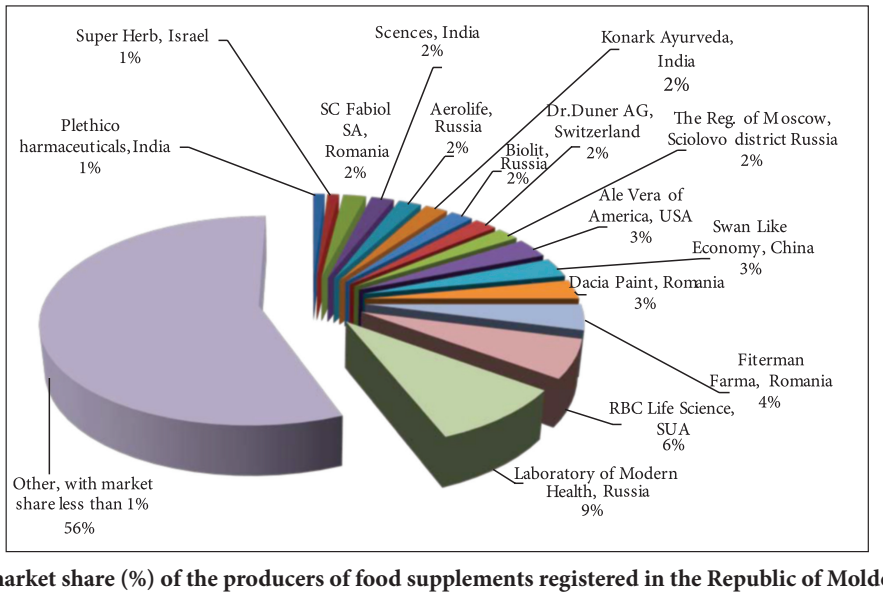


Fig. 1. The market share (%) of the producers of food supplements registered in the Republic of Moldova by 01.10.16

At the last stage of the statistical study of the national food supplements market we identified the share of the notified importers of the FS on the national market (tab. 3).

Table 3

Share of (%) of FS importers notified in the Republic of Moldova by 09.04.17

No	Importer	Market share, %
1.	IM Becor SRL	5
2.	Foralux	7
3.	DitaEstFarm	22
4.	Importers with quota 0 -0,5%	10
5.	Importers with quota 0,51 -1%	5
6.	Importers with quota 1.1-5%	41

Since the mandatory implementation of GMP compliance for medicinal products, more and more manufacturers choose to register their products as food supplements. Even after the expiring date of the Certificate of Registration of a medicine, the manufacturer chooses for the following registration to register their product as a food supplement.

This fact generated the so-called phenomenon of double registration of the same active substance on the national market. Thus, the chemical or plant substance with the same dose is found to be registered both, as a medicinal product and as a food supplement at the same time (tab. 4).

Table 4

Active substances with double registration status on the Republic of Moldova market

No	Name of the active substance	Presentation	Name of the medicine	Name of FS
1.	Silymarinum	140 mg	Lagosa	SameLive
2.	Pancreatinum	10000 IU	Kreon	DIGEX
3.	L- carnitinum	100 mg	Cartan	Proefect
4.	Glicinum	100 mg	Glicised	Glicin – Эвалар
5.	Carbo activatum	250 mg	Carbune activat	Toxinol

The substances listed in the table are just a few examples demonstrating the presence of this phenomenon on the national medicines and food supplements market. Operating with the Food Supplements Register it is very difficult to perform an analysis and statistical study in this area for the following reasons:

- The list is made in the format of a Microsoft Word file, which significantly complicates the statistical analysis.
- The same manufacturer or importer is registered in different cases under different names, eg., the same producer is registered as: "Laboratorul Sanatatii Moderne" (Modern Health Laboratory), "Laboratorul Sănătății Contemporane" (Contemporary Health Laboratory) and "Лаборатория Современного Здоровья" (Laboratory of Contemporary Health).
- The composition of food supplements included in the list of notified FS is specified in a superficial manner and without specifying doses. The composition is completely missing in the registered FS list. This is an obstacle in comparing the composition of food supplements and medicines.

Changing the status of their product from the medicine to a food supplement, the manufacturer pursues certain purposes, all of them having more or less an economic nature:

1. Avoiding the state control of different levels which is mandatory for the medicines – thus, avoiding additional costs and obtaining the possibility of saving on the quality of raw materials and finished products.
2. Avoiding the rigorous requirements imposed on medicinal products in the registration procedure and the presence of a detailed dossier including all the characteristics of the medicinal product. The lack of the requirement to declare sources of raw material, the technological process, the lack of the need to justify the validity - which again is done by producers in order to save and get financial benefits.
3. Possibility to make active and aggressive promotion of the product through the media, online space, through communication with medical personnel and pharmacists.
4. Including of food supplements in OTC list, which makes possible to sell them without a prescription, but only at the recommendation of the pharmacist or based on the advertising information.
5. Lack of vigilance and monitoring of the circulation of these products on the market, and the considerable decrease in the likelihood that the adverse reactions of these products will be considered or studied by the pharmacovigilance authorities.
6. Avoiding the need for post-marketing trials.
7. Mandatory implementation of GMP rules for medicines manufacturers.

These and many other factors influence the decision of manufacturers to change the status of their medicinal products in food supplements.

Inappropriate and abusive use of food supplements can lead to adverse health consequences for the population. Nonrational use of food supplements can also cause various side effects on all organ systems, generated both by the intrinsic effect of the food supplement and their association with medicines. Considering the fact that some food supplements are recommended to children and pregnant women and the elderly with chronic diseases, there is absolutely obvious need to regulate this area and monitor the circulation and consumption of these products by the population.

The pharmacist, being a major specialist in medicines, has the primary role both in implementing the necessary legislative changes and in counseling patients to prevent non-compliant use of food supplements.

Conclusions

1. Legislation on food supplements in the Republic of Moldova is incomplete and unsatisfactory formulated, which could be a prerequisite for the irrational use of food supplements with all subsequent consequences.
2. The value of the food supplements market is constantly increasing, making this area attractive from the economic point of view for both producers and distributors. The prevalence of economic interests over the medical interests may have adverse consequences for the health of the population.
3. The quality of food supplements on the national market is compromised once the most important market segments are held by manufacturers and importers for whom food supplements market is an auxiliary branch.
4. Frequent and obvious violations of the legislation provisions on the composition and labeling of food supplements are observed, and any provisions or reporting protocols on such breaches are missing.
5. The pharmacist has the primary role both in implementing the necessary legislative changes and in counseling patients to prevent the non-conformed use of food supplements.

References

1. Cohen PA. Hazards of hindsight – monitoring the safety of nutritional supplements. N Engl J Med. 2014;370(14):1277-80.
2. Vergari F, Tibuzzi A, Basile G. An overview of the functional food market: from marketing issues and commercial players to future demand from life space. Adv Exp Med Biol. 2010;698:308–21.
3. Ancuceanu R, Dinu M, Arama C. Weight loss food supplements: adulteration and multiple quality issues in two products of Chinese origin. Farmacia. 2013;63(1):28-43.
4. de Boer A, van Hunsel F, Bast A. Adverse food-drug interactions. Regul Toxicol Pharmacol. 2015 Dec;73(3):859-65.
5. Garcia-Cazarin ML, Wambogo EA, Regan KS, Davis CD. Dietary supplement research portfolio at the NIH, 2009-2011. J Nutr. 2014;144(4):414-8
6. Crişan O. Health claims in food supplement advertising. Farmacia. 2012;60(1):138-42.
7. Council of Europe. Homeostasis, a model to distinguish between foods (including food supplements) and medicinal products. Partial Agreement in the Social and Public Health Field Available from: http://www.coe.int/t/e/social_cohesion/soc-sp/Homeostasis%20(2).pdf.
8. European Parliament, Council of the European Union. Directive 2002/46/EC of 10th of June 2002 on the approximation of the laws of the Member States relating to food supplements. Official Journal of the European Communities, L 183/12.07.2002.
9. Garban G, Florescu N. Suplimentele alimentare: Ghid [Food Suppliments: Guide]. Bucharest: National Institute of Public Health; 2013. 37 p. Romanian.
10. Institute of Food Bioresources, Food Supplements Notification Office. List of notified food supplements according to Order no. 244/401/2005. Available from: http://www.bioresurse.ro/ documents/notificari244.pdf.
11. European Parliament, Council of the European Union. Regulation no. 1924/2006 of 20th of December 2006 on nutrition and health claims made on foods, Corrigenda, Official Journal of the European Union, L 12/18.01.2007.

DOI: 10.5281/zenodo.1186168
UDC: [616.211+616.216]-002.2-053.2

Cell therapy in the complex treatment of chronic rhinosinusitis in children

***Maniuc Mihail[1],** MD, PhD, Professor; **Ababii Polina[1],** MD, PhD, Associate Professor;
Danilov Lucian[1], MD, PhD, Professor; **Ghinda Serghei[2],** MD, PhD, Professor

[1]Department of Otorhinolaryngology, Nicolae Testemitsanu State University of Medicine and Pharmacy
[2]Department of Immunology and Allergy, Chiril Draganiuc Institute of Phtisiopulmonology
Chisinau, the Republic of Moldova

*Corresponding author: mihail.maniuc@usmf.md. Received October 29, 2017; accepted February 09, 2018

Abstract

Background: Determination of the general immune status and the effectiveness of the complex treatment with local immunostimulation (autologous mononuclear cells) of chronic rhinosinusitis in children.
Material and methods: The general immunity status and the effectiveness of the complex treatment with local immunostimulation were examined by the application in the maxillary sinuses of activated autologous mononuclear cells in 19 children with chronic rhinosinusitis and 116 healthy children.
Results: The presence of allergic reactions by increase in IgE and CD4/CD8 index was determined in patients examined. The sensitization to streptococcal antibodies was assessed by the cell sensitization data and the presence of streptococcal antibodies according to ASL-0 data. The inhibition of the T-cells was functional decrease of T-lymphocytes in the TTBL test with phytohemagglutinin and tendency towards a decrease of the T-CD-3 lymphocytes titer. The compensatory activation of the B-cells (increase in the IgA titer) was determined. The objective follow-up examination of the patients over one year revealed the absence of hyperemia and edema of the nasal mucosa, while an insignificant nasal obstruction was present in 5 children. The rhinomanometric examination demonstrated an increase in total volume indices and a decrease in the total nasal resistance in all treated children. The new method of local immunocorrection of chronic rhinosinusitis in children proved to be clinically effective.
Conclusions: There were determined increased IgE, CD4 / CD-8 index and CIC (PEG-8% with low molecular weight), cellular sensitization to streptococcal antibodies according to TTBL data, inhibition of the T-cells and tendency towards a decrease of T-CD-3 lymphocytes. Also, the compensatory activation of the B-cells (increase in the IgA titer) was determined.

Key words: chronic rhinosinusitis, children, cell therapy.

Introduction

Chronic rhinosinusitis in children is an important problem in otorhinolaryngology. The actuality of the study is conditioned by two factors: disease incidence and possible serious complications (middle ear and central nervous system complications, bronchopulmonary and other organs complications). Some bibliographic sources indicate that the prevalence of recurrent and chronic rhinosinusitis is over 15% of the population. Clikningham MJ, Chiu EJ, Landgraf JM, Gliklich RE [1] mentioned that the number of orbital complications in children constituted lately 12.4% of the total number of patients hospitalized with acute and chronic rhinosinusitis. This fact confirms that chronic paranasal rhinosinusitis has a high incidence and particular risks.

Nowadays due to certain factors, such as the increased resistance of the microbial flora to antibiotics, the continuous allergyzation of the population and the action of the environmental impurities, the drug therapeutics of these diseases is decreasing more and more. Therefore, researchers have expanded their search in other fields as well. The aggression on tissues causes changes in all processes which occur simultaneously in the body. Therefore, when speaking of the reparative regeneration, it is necessary to emphasize that both the general and local immunity are simultaneously affected in the impaired body area, being interdependent with all the physiological and pathological processes, including the restoration processes.

A priority direction in the field of pediatric otorhinolaryngology is the development of new methods of complex local or general immunostimulation therapy in order to restore nasal physiological functions in the case of chronic rhinosinusitis. This allows increasing the treatment effectiveness in children, to reduce complications and to improve the quality of their lives.

In clinical immunology, a new system of peptides of the immune system – cytokines, the secretion product of practically all the cells actively involved in immune processes, is intensely studied. These peptides are involved in inflammatory reactions, regulation of hematopoiesis, tissue regeneration processes, development of cellular and humoral immune reactions, infectious immunity, antitumor and transplantation immunity.

Depending on the nature of the tissue integrity disorders (skin, mucous membranes, connective tissue, vascular endothelium, etc.), the regulating action of cytokines can be directed to cells involved in inflammation (mononuclear and polymorphonuclear phagocytes, T-lymphocytes etc.), regeneration (fibroblasts, endothelial cells etc.), development of the immune response.

The cytokine action is performed according to the principle of complementarity – the information transmitted to

the cell is not only from an individual peptide, but from a complex of mutually stimulating regulatory cytokines, modulating the receptors on the surface of other mediators.

Autocells (MAPS – multipotent adult progenitor cells or MSC – mesenchimal stem cells) are a form of stem cells, considered one of the most acceptable grafts in cell therapy and tissue engineering. Currently there are technologies for obtaining stem cells from the peripheral blood. The advantages of using autocells are obvious: lack of immune conflict, reduced probability of patient`s contamination with hemotransmissible diseases, moral and ethical acceptability [3].

The new method of local immunocorrection (with autologous mononuclear cells) showed to be very effective in the conservative complex treatment of chronic compensated tonsillitis in children, the clinically positive effect serving as proof, the normalizing of the preimmune resistance status of the body (CD-16 content, normal antibodies, dynamics of total hemolytic complement activity and ESR), the obvious decrease in elevated indices of allergic reactions (eosinophils, IgE), the decrease in specific cellular sensitization levels to streptococcal and pneumococcal antigens, the increase in total lymphocyte content, the rise of levels and functional activity of T and B lymphocytes, the increase of cytokine profile efficiency, the reduction of the levels of proinflammatory cytokines (TNF-α, IL-8, IL-1β) and the growth of serum levels of anti-inflammatory cytokine (IL-4).

Sandul Al. [9] has demonstrated the advantages of the stimulated auto-lymphocytes action as a principle of correcting the local immunoresistence, which ensures the optimization of repair processes in the trepanation area after radical ear surgeries. The positive effect was accompanied by an increase in the absolute number of T-lymphocytes in the peripheral blood, the normalization of the immunoregulation index, the decrease in B-lymphocytes and in the content of different classes of immunoglobulins. The local use of auto-lymphocytes determines the positive onset of the postoperative period, accelerates the processes of inflammation eradication, increases the nonspecific immunoresistence, reduces numerically and attenuates the microbial population virulence, which, at the same time, becomes more sensitive to antibiotic therapy.

In connection with the above mentioned, we consider that the research of immune particularities in children with chronic rhinosinusitis is very important and constitutes a new direction in pediatric otorhinolaryngology from two perspectives: completing the knowledge of the disease pathogenesis and increasing the efficiency of the pathogenetic treatment, also serving as a basis to make a prognosis as accurately as possible. In this regard, we found it necessary to carry out a study on the cell therapy application in the treatment of recurrent and chronic rhinosinusitis.

Purpose of the research is to determine the general immune status and the effectiveness of the complex treatment with local immunostimulation (autologous mononuclear cells) of chronic rhinosinusitis in children.

Material and methods

The immunological examination group included 19 children with chronic rhinosinusitis and 116 healthy children.

Table 1

Distribution of patients by gender and age

Patients	No - 19
Boys	66.6%
Girls	33.3%
Age (years)	1112.0±2.3

By gender, the boys predominated in the examination group (2/3) (tab. 1), the girls constituted 1/3. The mean age was 12.0 ± 2.3.

All patients underwent complex immunological examination. The subpopulations of T and B lymphocytes (CD3, CD4, CD8, CD16, CD20) were determined by the Flow Cytometry method (Partec PAS I). To determine phagocytic cells, the phagocytic index and phagocytic number were used (Pavlovich S. A., 1998) [10]. The phagocytic activity of neutrophils was determined by the NBT (Nitro-Blue-Tetrazolium) test (B. H. Park et al., 1968). The content of circulating immune complexes was determined according to the procedure described by Grinevich I. A. and Kamenets L. I. [5] (Grinevich I. A. and Kamenets L. I. (1986) in the version adapted by S. Ghinda et al., (2008)). The Paull-Bunnell reaction was performed according to the procedure proposed by S. Ghinda (1984). Antistreptolysin-O, rheumatoid factor and C-reactive protein were determined by Humatex ASO, Humatex RF, Humatex CRP agglutination tests (manufacturer Human, Germany). The content of immunoglobulins A, G, M was determined by the solid-state immunoenzyme assay, using the reagents of OOK «Vektor BEST» (Russia), according to the attached instructions. Total IgE was estimated by the solid-state immunoenzyme assay using reagents (UBI Company), according to the attached instructions.

The local immunomodulatory therapy was performed by applying activated autologous mononuclear cells, derived from the patient's venous blood, into the maxillary sinuses after draining and removing the suppurating masses with 0.9% NaCl solution. The autologous cells were separated from the blood sample and activated by an original technology developed in the Laboratory of Tissue Engineering and Cell Culture of *Nicolae Testemitsanu* State University of Medicine and Pharmacy (concentration by gradient separation and centrifugation, then cultivation on special nutrient medium for 5 days in a CO_2 incubator).

The statistical analysis of the materials included operational methods of statistical assessment, including the Student criterion, the alternative variation, etc. (Leah P.E et al., 2006) and Windows 2007 computer operating system utilities.

Results and discussion

The analysis of immune changes in the recurrent and chronic rhinosinusal inflammatory process (according to the CIC titer of various molecular weights) demonstrated that CIC titers (PEG - 2.5%) and CIC (PEG - 4.2%) with high and medium molecular weight were higher in the group of children with rhinosinusitis, but with no significant statistical reliability. The CIC titre (PEG-8.0%) with low molecular weight was significantly higher in sick children compared to healthy subjects (p <0.001) (tab. 2).

Table 2

Characteristic of rhinosinusal intoxication in the study groups

Indices	Healthy children (n - 116)	Sick children (n - 19)
CIC (PEG-2.5%)	5.9±0.33	9.2±3.56
CIC (PEG-4.2%)	24.6±0.65	39.6±8.03
CIC (PEG-8.0%)	121±3.57	293±43.1○

Statistical veracity among studied groups: ○ – sick children and healthy children

Given the fact that immune complexes disappear from the body through the phagocytosis processes of macrophages, monocytes and neutrophils, the pre-immune resistance parameters were analyzed (tab. 3).

Table 3

Parameters of phagocytosis in the groups of examined children

Indices	Healthy children (n - 116)	Sick children (n - 19)
Phagocytic capacity of neutrophils (PhCN)	0.12±0.001	0.12±0.009
Phagocyte number (PhN)	72.2±0.76	71.8±2.83
Phagocyte Index (PhI)	4.1±0.70	4.8±0.77

Statistical veracity among the studied groups: ○ – sick children and healthy children

The ability to destroy engulfed bacteria (tab. 3), analyzed by the test of phagocytic capacity of neutrophils, demonstrated that the number of neutrophils involved in phagocytosis (PhN) and phagocytic index (PhI) in patients with rhinosinusitis and healthy children was equal. The pre-immune resistance parameters, such as phagocytosis, are the most relevant body defense mechanisms and change under exceptional circumstances.

The CD-16 lymphocyte titres (tab. 4) were similar in the groups of patients studied. The number of natural antibodies in children with recurrent and chronic rhinosinusitis was significantly lower than in healthy subjects (p<0.001). This phenomenon is accounted for the natural antibodies, which are the first protection and participate in the respective antigen inactivation.

Table 4

Parameters of pre-immune resistance

Indices	Healthy children (n - 116)	Sick children (n - 19)
CD-16	15.6±0.24	15.2±1.89
Natural antibodies	2.4±0.05	1.8±0.13○

Statistical veracity among studied groups: ○ – sick children and healthy children

Table 5

Allergic parameters

Indices	Healthy children (n - 116)	Sick children (n - 19)
IgE	9.2±0.27	57.9±22.79○
CD-4/CD-8	1.8±0.02	2.1±0.19

Statistical veracity among studied groups: ○ – sick children and healthy children

The analysis of some allergic parameters (tab. 5) in the groups of children examined showed a significantly higher IgE titer in the group of sick children (p<0.05). The CD-4/CD-8 immunoregulation index in children with rhinosinusitis was slightly higher. This fact demonstrates the presence of allergic reactions in children with recurrent and chronic rhinosinusitis.

The ASL-O titre (tab. 6) was significantly higher in sick children than in the control group. The CRP and RF titres did not show any difference between the studied groups.

Table 6

Titers of ASL-O, CRP and RF in the study groups

Indices	Healthy children (n - 116)	Sick children (n - 19)
ASL-O	11.5±2.08	178±96.5
CRP	1.1±0.20	1.22±0.66
RF	1.1±0.24	1.22±0.66

Statistical veracity among groups studied: ○ – sick children and healthy children

The sensitization of T-lymphocytes to streptococcal antigens (tab. 7) was significantly higher in sick children compared to the control group (p<0.001).

Table 7

Sensitization of T-lymphocytes to streptococcal antigens

Indices	Healthy children (n - 116)	Sick children (n - 19)
TTBL-streptococcus	1.3±0.07	3.3±0.46○
TTBL- staphylococcus	1.6±0.06	2.1±0.29

Statistical veracity among groups studied: ○ – sick children and healthy children

The sensitization of T-lymphocytes to staphylococcal antigens in patients with rhinosinusitis was relatively higher compared to the control group, but with no statistical difference.

Table 8

Quantitative and functional parameters of T-lymphocytes

Indices	Healthy children (n - 116)	Sick children (n - 19)
TTBL-PHA	73.7±0.41	61.4±1.21○
CD-3	70.0±0.43	66.3±2.89
CD-4	44.0±0.31	46.3±1.47
CD-8	24.5±0.30	23.1±1.99

Statistical veracity among groups studied: ○ - sick children and healthy children

The T-lymphocytes functional activity (tab. 8), assessed by the (TTBL-PHA) blast transformation of lymphocytes by phytohemagglutinin, was lower in sick children compared to healthy ones ($p<0.001$). The T-lymphocytes (CD-3) titre was low in patients with the rhinosinusal disease, but with no statistical difference. The T-helper (CD-4) lymphocytes were higher, while the T-suppressor lymphocytes (CD-8) were lower in sick children than in healthy ones. This suggests the suppression of T-lymphocytes in sick children.

Table 9

Quantitative and functional parameters of B-lymphocytes

Indices	Healthy children (n - 116)	Sick children (n - 19)
CD-20	8.0±0.14	6.0±1.20
IgG	10.6±0.27	10.2±0.59
IgA	1.1±0.03	1.9±0.25○
IgM	1.0±0.02	1.2±0.12

Statistical veracity among groups studied: ○ - sick children and healthy children

The B-lymphocytes count (CD-20) in sick and healthy children did not differ. No differences in IgM and IgG were found in the studied groups. A significant increase in IgA ($p<0.001$) was determined in the group of children with chronic rhinosinusitis, resulting in the activation of B-cells (tab. 9).

At hospitalization, patients had unilateral or bilateral nasal obstruction in 19 cases (100%). The patients presented with purulent nasal discharge – 10 cases (52.6%) and mucopurulent nasal discharge – 9 (47.3%) cases. Quite often, children`s parents reported snoring in 12 cases (63%), and fever or subfebrility during a sinusitis relapse in 13 cases (68.4%). Of major symptoms, headache was present in 100% cases, hypo/anosmia was determined in 9 cases (47.3%). The endoscopic examination revealed changes in the inferior nasal turbinate in 8 cases (42.1%) and various external modifications in the middle nasal turbinate in 11 cases (57.8%). The radiological investigations of the nose and paranasal sinuses revealed a total or partial opacity in the study groups: maxillary sinus – 19 cases (100%), ethmoidal sinus – 12 cases (63.1%) and frontal sinus in 5 cases (26.3%).

The rhinomanometry results were compared between the children with recurrent and chronic rhinosinusitis and the control group. The children in the study group had reduced total volume of the nasal fossa compared to the control group ($P<0.05$). An increase was attested in the total resistance at 150 Pa ($P<0.05$) in the study groups.

The disease course and treatment outcomes of patients in the study group were assessed over one year. The objective examination determined mucosal changes in only three patients (15.7%): hyperemia and edema of the pituitary and pale or violet turbinates. This can be accounted for the allergic and immunologic status changes in these children. The most common clinical sign was nasal obstruction – 5 cases (26%). Headache was rarely revealed – 3 cases (15.7%). Of minor signs, cough, snoring and fever or subfebrility were practically not detected. The rhinomanometric examination demonstrated an increase in the total volume indices and a decrease in the total nasal resistance in all treated children.

Conclusions

1. According to the data received on pre-immune resistance, the increase in CIC (PEG-8% with low molecular weight) confirms the presence of the body intoxication signs.
2. The presence of allergic reactions in examined patients was determined by the increase in IgE and the CD4 / CD-8 index.
3. The sensitization of streptococcal antibodies according to TTBL data (cell sensitization) and the presence of streptococcal antibodies according to ASL-O data (humoral sensitization) were assessed.
4. The inhibition of T-cells (according to data on T-lymphocyte functional decline in TTBL test with phytohemagglutinin and tendency of T-CD-3 lymphocytes towards a decrease) was revealed.
5. The compensatory activation of B-cells (increase of IgA) was determined.
6. The new method of local immunocorrection (with autologous mononuclear cells) in the conservative complex treatment of chronic rhinosinusitis in children showed to be effective, demonstrating a clinically positive effect.

References

1. Sedaghat AR, Phipatanakul W, Cunningham MJ. Prevalence of and associations with allergic rhinitisin children with chronic rhinosinusitis. Int J Pediatr Otorhinolaryngol. 2014;78(2):343-7. doi:10.1016/j.ijporl.2013.12.006. PubMed Central PMCID: PMC3966022.
2. Brietzke SE, Shin JJ, Choi S, Lee JT, Parikh SR, Pena M, et al. Clinical consensus statement: pediatric chronic rhinosinusitis. Otolaryngol

Head Neck Surg. 2014;151(4):542-53. doi:10.1177/0194599814549302. PubMed PMID: 25274375.
3. Cunningham MJ, Chiu EJ, Landgraf JM, Gliklich RE. The health impact of chronic recurrent rhinosinusitis in children. Arch Otolaryngol Head Neck Surg. 2000;126(11):1363-8.
4. Danilov L. Amigdalita cronică la copii [Chronic tonsillitis in children]. Chisinau: Medicina; 2014. 210 p. Romanian
5. Danilov L. Particularităţile florei microbiene în patologiile inflamatorii acute şi cronice orofaringiene la copii [Peculiarities of microbial flora in acute and chronic inflammatory oropharyngeal pathologies in children]. Buletin de perinatology (Chisinau) [Bulletin of perinatology]. 2014;61(1):55-61. Romanian.
6. Ababii I, et al. Optimizarea regenerării reparatorii a ţesuturilor şi imunogenezei locale în contextul funcţionarii nanosistemelor naturale [Optimization of regenerative tissue reconstruction and local immunogenesis in the context of the natural nanosystems functioning]. Chisinau: Medicina; 2011. 335 p. Romanian.
7. Koval'chuk LV, Gankovskaia LV. Immunotsitokiny i lokal'naia immunukorrektsiia [Immunocytokines and local immunocorrection]. Immunologiia [Immunology] (Russia). 1999;(1):4-7. Russian.
8. Danilov L. Aspecte etio-patogenetice şi optimizarea tratamentului conservator în amigdalita cronică la copii [Etiopathogenetic aspects and optimization of conservative treatment of chronic tonsillitis in children] [dissertation]. Chisinau: "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova; 2016. 274 p. Romanian.
9. Sandul A. Mastoidoplastia cu autoos spongios combinată cu imunocorecţie pentru tratamentul bolnavilor cu otite medii cronice suppurate [Mastoidoplasty with an autologous cancellous bone in combination with immunocorrection in the treatment of patients with chronic purulent otitis media] [dissertation]. Chisinau: "Nicolae Testemitanu" State University of Medicine and Pharmacy of the Republic of Moldova; 1996. 265 p. Romanian.
10. Pavlovich SA. Osnovy immunologii [Fundamentals of immunology]. Minsk: Vysheishaia shkola; 1998. 114 p. Russian.
11. Park BH, Fikrig SM, Smithwick EM. Infection and nitroblue-tetrazolium reduction by neutrophils. Lancet. 1968;2(7567):532-4.
12. Grinevich IuA, Kamenets LIa. Osnovy klinicheskoi immunologii opuholei [Fundamentals of clinical immunology of tumors]. Kiev: Zdorov'ia; 1986. 158 p. Russian.
13. Ghinda S, Sofronie S, Chiroşca V, Privalova E, Calenda O, Smeşnoi V, Lesnic E, Barbuţa A, Rotaru N, Iaconi L, inventors. Metoda concomitenta de determinare a CIC cu masa moleculară joasa, medie şi nalt [Concomitant method of determining CIC with low, medium and high molecular weight] [Certificate of innovation no. 46 registered at "Chiril Draganiuc" Phthisiopneumology Institute of the Republic of Moldova. 2008 May 12]. Romanian.

DOI: 10.5281/zenodo.1186182
UDC: 616.24-002.5-085:615.015.8

Markers of the oxidative stress and antioxidant system in pulmonary drug susceptible and drug resistant tuberculosis

*Lesnic Evelina[1], MD, PhD, Associate Professor, Gudumac Valentin[2], MD, PhD, Professor

Department of Pneumophtisiology, Nicolae Testemitsanu State University of Medicine and Pharmacy
Department of Biochemistry, Laboratory of Clinical Biochemistry, Chisinau, the Republic of Moldova

*Corresponding author: evelina.lesnic@usmf.md. Received December 22, 2017; accepted February 16, 2018

Abstract

Background: The oxidative stress biomarkers in tuberculosis were studied, but the differences between the pulmonary drug susceptible and drug resistant forms were not identified.

Material and methods: A prospective, case-control study, which included 51 patients, distributed in 2 groups: the 1st study group (N=24 new cases with drug susceptible tuberculosis) and the 2nd group (N=27 new cases with MDR-TB) similar distributed according to the sex and age were compared with a control group constituted from 36 healthy persons. The intensity of the oxidative stress was appreciated through the serum concentration of the advanced oxidation protein products, lysosomal marker N-acetil-β-D-glucosaminidase, advanced glycation end-products (AGE_s). The antioxidant defense was assessed through the total serum antioxidant activity, the activity of the glutathione enzymes and proteins with antioxidant role.

Results: In MDR-TB carbohydrate peroxidation biomarker versperlysines-like AGE_s was diminshed, as well as the serum antioxidant defense assessed through CUPRAC method. In drug susceptible tuberculosis was established the elevation of the protein peroxidation, high lysosomal membrane damage and increased acute phase protein – ceruloplasmine. Antioxidant enzyme glutathione S-transferase had lower activity in both types of tuberculosis which contributed to the increasing of the γ-glutamyltransferase.

Conclusions: The oxidative stress level was more elevated in drug susceptible tuberculosis and antioxidant defense was more impaired in the MDR-TB group. The antioxidant biomarker – glutathione S-transferase activity, was lower in both types of tuberculosis which increased the γ-glutamyltransferase activity. The polymorphism assessment of the glutathione S-transferase enzyme is important for the individualized therapy and reducing the toxicity of the anti-tuberculosis treatment.

Key words: tuberculosis, oxidative stress, antioxidant system.

Introduction

Tuberculosis evolution and treatment response are determined by the mycobacteria virulence, protective mechanisms and the organism's capacity to fight against the aggression of the oxidative stress through the antioxidant defense [3]. The oxidative stress (OS) is caused by the imbalance between the systemic manifestation of the reactive oxygen species (ROS) and the biological system's ability to detoxify them and to repair the resulting damage [7]. ROS are produced from molecular oxygen following the normal cellular metabolism. As well as, numerous exogenous factors are the sources of the ROS: smoking, ozone exposure, hyperoxia, ionizing radiation, intoxication with heavy metals and some drugs. ROS are divided into 2 groups: free radicals and non-radicals. From the group of the free radicals there are: O_2^- (superoxide radical), OH^-(hydroxyl radical), H_2O_2 (hydroxyl peroxide), ROOH (organic hydroperoxide), RO^- and ROO^- (alkoxy and peroxy radicals), HOCl (hypochlorus acid) and $ONOO^-$ (peroxynitrite) [22]. The biggest physiological damage determines superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydroxyl peroxide (H_2O_2). At the biochemical level severe OS lowers the effectiveness of the enzymatic and non-enzymatic antioxidant defense [7]. The disturbances in the normal redox system cause toxic effects through the peroxidation of the cellular DNA, proteins, lipids, carbohydrates and other biological macromolecules [22]. Mild OS causes cell apoptosis and severe OS– necrosis and functional impairment. Many pathological disturbances and diseases are attributed to the OS: cancer, atherosclerosis, hyperthension, respiratory distress syndrome, chronic obstructive pulmonary disease, asthma, pulmonary fibrosis and neurological disturbances [6, 8, 18, 23]. Advanced oxidation protein products (AOPP) are considered novel markers of the OS [5]. They result from the interaction between the chlorine oxidants (chloramines and hypochlorus acid) with plasmatic proteins [26]. AOPP are carried by oxidized plasma proteins, especially albumin, are excreted by the kidneys and the highest concentrations were identified in patients with severe chronic renal failure, hyperparathyroidism and continuous treatment with calcium and vitamin D [24]. High concentrations of the AOPP are correlated with high level of the advanced glycation end products (AGE_s), resulting from the non-enzymatic glycation of the proteins, lipids and nucleic acid, following the "Maillard" chain of reaction [10]. High AGE_s concentration is identified in patients with diabetes mellitus and represents a marker of the hyperglycemia, non enzymatic glycosilation of the proteins and excessive activation of the polyol way [18]. Close correlation was found between high levels of AOPP and AGE_s in monocyte-mediated inflammatory processes [3].

Lysosomal enzymes are mediators of the tissue damage in any type of inflammation [3].

N-Acetyl-β-glucosaminidase (NAG) is a high molecular-weight (~140 kDa) hydrolytic lysosomal enzyme, iden-

tified in many tissues. It breaks chemical bonds of glycosides and amino sugars that form structural components of the tissues and is localized in various parts of the cell, including the cell membrane. There are two main isoenzymes: isoenzyme-A and isoenzyme-B, being different in their heat sensitivity and stability. Isoenzyme-A is contained in the azurophilic granules of polymorphonuclear leukocytes and is excreted during the exocytosis. Isoenzyme-B is localized in the lysosomal membrane and is excreted during lysosomal damage [25]. High activity of NAG is a marker of tissue damage, inflammation and loss of lysosomal integrity [25].

The antioxidant system is composed by the hydrophilic antioxidant compounds identified in the cytoplasm and blood serum, as well as the hydrophobic compounds, which are localized in the biological membranes [7]. Gluthatione (GSH) is the most important antioxidant in animals, plants and bacteria. It prevents the damage of the cellular components produced by ROS and heavy metals. GSH reduces disulfide bonds formed within cytoplasmic proteins and cysteines serving as an electron donor. In the redox process the GSH is converted in its oxidized form, called glutathione disulfide (GSSG.) Once oxidized, the GSH can be reduced back by glutathione reductase, using NADPH as an electron donor. The ratio between reduced glutathione (GSH) to oxidized glutathione (GSSG) within cells is used as a measure of the cellular oxidative stress [19]. Glutathione S-transferase (GST) is a cytosolic, mitochondrial and microsomal enzyme, involved in the detoxification of the xenobiotics through the conjugation catalysis of the reduced glutathione in mercapturic compounds [11]. Assessment of the GST activity is a diagnostic tool of the OS and efficiency of the detoxification mechanisms related to GST [1, 14, 16]. The *γ*-glutamyltransferase (*γ-GPT*) is a key-enzyme of the *γ*-glutamyl cycle, which catalyses the transfer of the *γ*- glutamyl group from the glutathione to other amino acids [1, 4]. It is localised in the cell's membrane, and the enzyme's active centre at the exterior cell's border. It has a major role in the detoxification of the inflammatory mediators (cytokines, acute phase proteins), carcinogenic substances and toxine [3]. The synthesis of the *γ-GPT* is induced by drugs, colestasis, alcohol consumption, hepatic tumors and cirrhosis [1]. Actually, the *γ-GPT* is considered advanced biomarkers of the OS [9].

Drug susceptible TB is treated with first line anti-tuberculosis drugs: isoniazid, rifampicin, ethambutol, pirazinamide and streptomycin [27]. Tuberculosis determined by the multidrug resistant strains (MDR-TB) is treated during 18-24 months with 2nd line antituberculosis drugs according to the drug susceptibility test [28]. The standard treatment for MDR-TB consists in injectable antibiotics – aminoglycozides (kanamycin, amikacin or capreomycin) and orally administrated anti-tuberculosis drugs: fluoroquinolones (levofloxacin, moxifloxacin or gatifloxacin), ethionamide, prothionamide, paraaminosalicylic acid and cycloserine) [28]. The rate of adverse drug events, usually correlated with the OS is much higher in patients treated with second line anti-TB drugs, patients with TB-HIV co-infection, than in those treated with first line drugs [12, 26]. The aim of the study was the assessment of the oxidative stress biomarkers and antioxidant system compounds in patients with pulmonary drug susceptible and drug resistant tuberculosis and their comparison with a group of healthy persons.

Material and methods

It was realized a prospective research evaluating the biochemical markers of the OS in 87 cases, from which 24 were new cases with drug susceptible pulmonary tuberculosis included in the 1st study group and 27 were MDR-TB patients which were included in the 2nd study group. The groups were compared between them and were compared with a control group (CG) composed from 36 healthy persons assessed according to the clinical and biochemical criteria. The research reported ethics committee approval (nr. 14 of 21/11/2017) and patients' consent was obtained. Patients were diagnosed in the medical specialized institutions of Chisinau during the period 01.01.2016-31.08.2016. Included criteria in the study group were: age more than 18 years old, patient diagnosed with pulmonary tuberculosis, new case type, the diagnosis confirmed through the conventional microbiological methods (microbiological examination and molecular genetic test of the sputum). The study investigation schedule included information about sex, age, radiological aspects, microbiological patient's status, treatment regimen and adverse drug reactions. The included criteria in the control group were: age more than 18 years old, conditioned healthy persons according to the clinical examination, blood test (complete blood count) and biochemical tests (liver transaminases, bilirubin test, hepatitic virus serological tests, HIV serology).

The biochemical investigation of patients was performed during the intensive phase of the treatment. The 36 healthy persons from the control group were investigated in ambulatory conditions. The estimation of the biochemical indices in the serum was performed using the methods with microquantities of the evaluated material. The samples were analysed by spectrophotometry in the maximum standardization conditions. Total proteins were determined using the Lowry modified method [9]. The AOPP were analysed according to the Witko-Sarsat V. modified method [24]. The AGEs were quantified in two types: pentosidine-like AGEs and vesperlysines-like AGEs [17, 21]. The micromethod was based on the fluorescence measure of the intensity of the samples diluted in the phosphate tampon at λexc 335 nm, λem 385 nm (quantification of the pentosidine-like AGEs) and at λexc 370 nm, λem 440 nm (quantification of the vesperlysines-like AGEs) according to the Sero L. modified method [10, 20].

The determination of the total antioxidant activity (tAOA) was performed using two procedures: 1) method based on the degradation of the 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical at the interac-

tion with serum compounds with the antioxidant properties and measure of the decreasing absorbance at 734 nm [9]; 2) method CUPRAC (Cupric Ion Reducing Antioxidant Capacity) based on the reducing capacity of the cupric ion through the captation of the hydroxyl radical [20].

The serum antioxidant capacity was assessed by the dosage of the glutathione enzymes (GST and γ-GTP) activity using the analysis kits of the Eliteh (France) producer, according to the attached instructions.

Statistical analysis was carried out by the comparative assessment of the quantitative and qualitative peculiarities of the selected patients using the Microsoft Excel XP programme. Accumulated material was systematized in simple and complex groups. For the testing of significant differences between the studied indices of the compared samples it was performed the statistic non-parametric t test and the significance threshold $p<0.05$.

Results

By distributing patients according to the biological characteristics was established a similar rate of men and women in all three groups, with the predomination of men in the same proportion, which permitted the comparability of the results. The same proportion of young persons, aged less than 44 years old, was established in all groups. All enumerated conditions permitted the comparability of the laboratory data (tab. 1).

Table 1

Segregating patients in sex and age groups

Biological segregation	parameters	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		N (%)	N (%)	N (%)
Sex stratification	Men	14 (58%)	18 (67%)	24 (67%)
	Women	10 (42%)	9 (33%)	12 (33%)
Stratification in age groups	18-44 years	18 (75%)	21 (77%)	29 (81%)
	≥45 years	6 (25%)	5 (23%)	7 (19%)

Detected by passive way, using standard tools (microbiological examination and chest X-ray) for the investigation of the symptomatic patients were 15 (62%) cases of the 1st SG and 17 (63%) cases of the 2nd SG. The main proportion of both study groups was constituted from the patients with pulmonary infiltrative TB: 22 (91%) in the 1st SG and 24 (89%) in the 2nd SG. Radiological investigations identified lung destruction in all selected TB patients. Microbiological status was positive in all patients and drug susceptibility testing permitted their distribution according to the obtained drug resistance results. Standard treatment for drug susceptible TB was administrated in patients from the 1st SG and standard treatment for MDR-TB in patients from the 2nd SG. There were no major adverse drug reactions identified in the selected patients.

Biomarkers of the OS constituted the blood concentration of the AOPP, the activity of the NAG, the concentration of the pentosidine-like AGEs and vesperlysines-like AGEs. Assessing the concentration of the AOPP was established a statisticaly higher level in the 1st SG compared with the CG ($p<0,05$) and unsignificantly higher concentration in the 2nd SG comparated with the CG. No differences were established between the study groups. The NAG activity was significantly higher in the 1st SG compared with the 2nd SG, as well as compared with the CG. In the 2nd SG the NAG activity was similar with the results obtained for the CG. The concentration of the pentosidine-like AGEs was significantly lower in the 1st SG compared with the 2nd SG and compared with the CG. The level of vesperlysines-like AGEs was significantly lower in the 2nd SG compared with the 1st SG and compared with the control group ($p<0,001$). Data are shown in the table 2.

The antioxidant defense was evaluated through the glutathione-related enzymes, non-enzymatic proteins and total antioxidant activity (tAOA) of the serum. The serum level of the glutathione-S-transferase (GST) was significantly lower in both study groups compared with the control group at the same statistical level. The activity of γ-glutamyltranspeptidase (γ-GPT) was significantly increased in both study groups in comparison with the control

Table 2

Indices of the oxidative stress

Oxidative system	Parameter	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		M±SE (%)	M±SE (%)	M±SE (%)
Proteic peroxidation	*AOPP* μMol/l	45.07±22,15(131%) □	38.42±15,77(111%)	34.349±3,58 (100%)
	NAG mMol/s.L	87.79±42.17(133%)■□	66.29±22.27(101%)	65.88±18.63(100%)
Carbohydrate peroxidation	*Pentosidine-like AGEs*	140,79±73,63(66%) ○	216,56±151,64(100,8%)	208,5±16,27(100%)
	Vesperlysines-like AGEs	469,89±166,13(93%)○●	273,82±106,83(62%) ◊	343,2±49,63(100%)

Note: AOPP – advanced oxidation protein products; NAG – N-acetyl-β-D-glucosaminidase; AGEs – Advanced glycated end-products. The percentage was assessed comparing the study groups with the reference value of the control group (100%). Comparison between study groups ● $p<0.001$ ▪ $p<0.05$; comparison between the 1st SG and CG ○ $p<0.001$ □ $p<0.05$; comparison between the 2nd SG and CG ◊< 0.001.

Table 3

Indices of the antioxidant defense

Antioxidant system	Parameter	1st SG (N=24)	2nd SG(N=27)	CG (N=36)
		N (%)	N (%)	N (%)
Glutathione-related enzymes	Glutathione S-transferase nMol/s. L	16.650±5,98 (77%) ○	16.222±8,52 (75%) ◊	21.5±6.75 (100%)
	γ-Glutamyltransferase U/l	62.95±6.56 (147%)Δ	78.24±3.58 (183%)●	427±7.02 (100%)
Total antioxidant activity (tAOA)	Method ABTS mMol/l	0.65±0.03 (91%)	0.67±0.03 (94%)	0.71±0.004 (100%)
	Method CUPRAC mMol/l	1.42±1.49 (276%) Δ●	0.59±0.47 (88%)	0.52±0.04 (100%)
Non-enzymatic antioxidants	Ceruloplasmine mg/ml	911.31±210.70 (125%) □	852.101±256.1 (117%)	724.3±27.8 (100%)
	Total protein	59.21±4.95 (103%)	59.53±2.18 (103%)	57.1±2.3 (100%)

Note: The percentage was assessed comparing the study groups with the reference value of the control group (100%). Comparison between study groups ● p<0,001 ▪ p<0.05; comparison between the 1st SG and CG Δ p<0.001○ p<0.01 □ p<0.05; comparison between the 2nd SG and CG ◊< 0.01.

group. The index was significantly higher in the 2nd SG compared with the 1st SG. The total antioxidant activity (tAOA) of the serum assessed through the ABTS and CUPRAC methods established different results. tAOA evaluated using ABTS method was nonsignificantly lower in both study groups compared with the CG, without differences between groups. Assessing tAOA using CUPRAC method, was identified a significantly higher level in the 1st SG and a mild decreasing tendency in the 2nd SG compared with the CG. Comparing the groups of patients it was identified a statistically higher level of the tAOA using CUPRAC method in the 1st SG compared with the 2nd SG. The concentration level of the ceruloplasmine, known as a protein of the acute phase, with antioxidant role was detected significantly higher in the study groups compared with the CG. The concentration of the total serum proteins (albumin, α1, α2, β and γ globulins), which include and those with an antioxidant role was lower in CG compared with both study groups. No differences were identified between study groups (tab. 3).

Discussion

Distributing the patients, according to the sex and age, it was determined the predomination of the men at reproductive age (18-44 years) in both study groups, as well as in the control group, which allowed the comparability of the results.

Two thirds of both study groups were detected by using standard microbiological examination and chest X-ray investigation. Similar data were obtained in the national studies. The majority of both study groups was diagnosed with pulmonary infiltrative TB with lung destruction. Microbiological status was positive in all patients and drug susceptibility testing permitted their distribution according to the obtained drug resistance results. Standard treatment for drug susceptible TB was administrated in patients from the drug susceptible group and standard treatment for MDR-TB in patients from the 2nd SG. The regimens were used according to the WHO recommendations [27, 28].

The biomarker of the OS – the concentration of the AOPP was higher in the drug susceptible TB group, compared with the MDR-TB group and healthy group demonstrating a higher level of the oxidative damage and protein catabolism in the 1st SG. In the speciality literature, comparable studies about AOPP concentration in patients with drug susceptible and MDR-TB have not been identified. The serum concentration of the pentosidine-like advanced glycation end-products determined a significantly lower concentration in patients with drug susceptible tuberculosis compared with the patients with MDR-TB and healthy group. The results confirmed the state of organism's starvation and exacerbation of catabolic processes assessed through the AOPP dosage. The serum level of vesperlysines-like advanced glycation end-products was much lower in the group of MDR-TB patients, compared with the drug-susceptible group. Both biomarkers of the carbohydrate peroxidation demonstrated the disturbances in the glycemic metabolism, which cannot be compared with the data issued from the international studies where enrolled diabetic patients with tuberculosis were. The high activity of the NAG is considered a biomarker of the xenobiotic-induced lysosomal membrane damage. The activity level was significantly higher in the drug susceptible tuberculosis group compared with the patients with MDR-TB and healthy group. It demonstrated the sustained oxidative damage of the leukocytes by the acute inflammatory process. No similar studies were found in the international review. The biomarker of the antioxidant defense – total antioxidant activity assessed using ABTS method was mildly decreased in the groups of tuberculosis-patients; on the other hand, CUPRAC method established contradicto-

ry results. So, in the drug susceptible group AOA was three times higher and in MDR-TB group it was slightly decreased compared with the healthy group. It could be explained by different components of the serum involved in the antioxidant defense identified through the ABTS and CUPRAC methods. The concentration of the ceruloplasmine, acute phase proteine with antioxidant role, was higher in the drug susceptible group compared with the MDR-TB and control group, and confirmed a higher toxic damage due to acute inflammatory process, as did the lysosomal marker (NAG), ceruloplasmine and advanced oxidation protein products.

The activity of the glutathione S-transferase enzyme (GST) was significantly diminished in the group of patients with tuberculosis. The literature data correlated the epigenetic disturbances with the low level of the GST gene expression, especially of the isoformes associated with the hepatotoxicity of the anti-tuberculosis drugs [15]. In consequence, the level of γ- glutamyltransferase at patients with both types of tuberculosis was significantly increased.

Conclusions

1. In patients with drug susceptible tuberculosis was established the increased level of the protein peroxidation, lysosomal membrane damage and acute phase protein.
2. In patients with MDR-TB were identified severe disturbances of the carbohydrate peroxidation evaluated through the verspelysines-like AGEs and reduced antixodant defense assessed through CUPRAC method.
3. Enzymatic antioxidant defense – glutathione S-transferase activity, was lower in both types of tuberculosis which contributed to the increasing of the γ- glutamyltransferase during the antituberculosis treatment.
4. The polymorphism assessment of the glutathione S-transferase enzyme is important for the individualized therapy and reducing the toxicity of the anti-tuberculosis treatment.

References

1. Andronache L. Protocoale standardizate de cercetare a metabolismului glutationic [Standardized research protocols of glutathione metabolism]. Chisinau; 2014. Romanian.
2. Apak R, Güçlü K, Özyürek M, et al. Total antioxidant capacity assay of human serum using copper(II)-neocuproine as chromogenic oxidant: the CUPRAC method. Free Radic Res. 2005;39(9):949-61.
3. Abbas A, Lichtman A, Pillai S. Basic immunology: Functions and disorders of the immune system. Philadelphia: Elsevier; 2015. 352 p.
4. Arshya B, Nirmaladevi K, Deepalarhmi P, et al. Serum ceruloplasmin albumin ratio as a biochemical marker to assist the diagnosis, treatment and prognosis of pulmonary tuberculosis patients. Natl J Basic Med Sci (India). 2014;6(1):2-5.
5. Andreozzi R, Caprio V, Insola A, et al. Advanced oxidation processes (AOP) for water purification and recovery. Catalysis Today. 1999;53(1):51-9.
6. Bargagli E, Olivieri C, Bennett D, et al. Oxidative stress in the pathogenesis of the diffuse lung disease. Respir Med. 2009;103(9):1245-56.
7. Birben E, Sahiner UM, Sackesen C, et al. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19.
8. Comhair SA, Erzurum SC. Antioxidant responses to oxidant-mediated lung diseases. Am J Physiol Lung Cell Mol Physiol. 2002;283(2):L246-55.
9. Gudumac V, Niguleanu V, Caragia S, Tagadiuc O, Vartician A. Investigatii biochimice [Biochemical investigation]. Chisinau: Elena V.I. SRL; 2008. 72 p. Romanian.
10. Gudumac V, Rivneac V, Tagadiuc O, et al. Metode de cercetare a metabolismului hepatic: Elaborare metodica [Methods of research of hepatic metabolism]. Chisinau: [publisher unknown]; 2012. 162 p. Romanian.
11. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: the first enzymatic step in mercapturic acid formation. J Biol Chem. 1974;249(22):7130-9.
12. Kehinde AO, Adaramoye O. Biochemical changes in blood and tissues of rats following administration of anti-tuberculosis drugs. Afr J Biochem Res. 2015;9(4):67-72.
13. Lesnic E, Ustian A, Niguleanu A, Malic A, Paladi C. Social features of patients with pulmonary tuberculosis. Туберкулез, легеневі хвороби, ВІЛ-інфекция [Tuberculosis, lung diseases, HIV infection] (Kiev). 2016;25(2):36-40.
14. Lesnic E, Paladi C, Niguleanu A, Ciubotaru V, Sirbu P, Curocichin Gh. Segregation of tuberculosis patients by social, demographic and economic features on the model of Chisinau city and the role of the community support. Curierul Medical (Chişinău). 2016;59(4):11-7.
15. Todoriko LD, Semianiv IO, Lesnic EV. [Analysis of the GSTT-1 gene polymorphism in patients with tuberculosis with regard to the version of Mycobacterium tuberculosis resistance]. Bukovins'kii medichnii visnik [Bukovinian Medical Herald]. 2016;20(2(78)):169-71. Ukrainian.
16. Liu F, Jiao AX, Wu XR, et al. Impact of glutathione S-transferase M1 and T1 on antituberculosis drug-induced hepatotoxicity in Chinese pediatric patients. PLoS One. 2014;9(12):e115410.
17. Makita Z, Vlassara H, Cerami A, Bucala R. Immunochemical detection of advanced glycosylation end-products in vivo. J Biol Chem. 1992;267(8):5133-8.
18. Nowortny K, Jung T, Hohn A, et al. Advanced glycation end-products and oxidative stress in type 2 diabetes mellitus. Biomolecules. 2015;5(1):194-222.
19. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med. 2001;30(11):1191-212.
20. Séro L, Sanguinet L, Blanchard P, et al. Tuning a 96-Well Microtiter plate fluorescence-based assay to identify AGE inhibitors in crude plant extracts**.** Molecules. 2013;18(11),14320-39.
21. Van Zoelen MA, Wieland CW, van der Windt GJ, et al. Receptor for advanced glycation end-products is protective during murine tuberculosis. Mol Immunol. 2012;52(3-4):183-9.
22. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.
23. Valko M, Rhodes CJ, Moncol J, et al. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160(1):1-40.
24. Witko-Sarsat V, Friedlander M, Capeillère-Blandin C, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney Int. 1996 May;49(5):1304-13.
25. Wen X, Kellum J. N-Acetyl-beta-D-Glucosaminidase (NAG). In: Vincent JL, Hall JB, editors. Encyclopedia of intensive care medicine. Berlin: Springer; 2012
26. Wiid I, Seaman T, Hoal EG, et al. Total antioxidant levels are low during active TB and rise with anti-tuberculosis therapy. IUBMB Life. 2004;56(2):101-6.
27. World Health Organization. Guidelines for treatment of drug-susceptible tuberculosis and patient care. Geneva: WHO; 2017. 80 p.
28. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis. Geneva: WHO; 2014. 447 p.

Declaration of conflict of interests. Nothing to declare.

DOI: 10.5281/zenodo.1186184
UDC: 615.225.2.035.1

Selection of antihypertensive drugs from the perspective of clinical pharmacology

Ghicavii Victor, MD, PhD, Professor, Corresponding Academician;
Bacinschi Nicolae, MD, PhD, Professor; **Podgurschi Lilia,** MD, PhD, Associate Professor;
***Turcan Lucia,** MD, PhD, Associate Professor; **Chianu Marin,** MD, Assistant Professor

Department of Pharmacology and Clinical Pharmacology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
*Corresponding author: lucia.turcan@usmf.md. Received January 12, 2018; accepted February 26, 2018

Abstract

Background: The rational use of medicines remains one of the most important directions of public health, especially in socio-medical diseases, including arterial hypertension.

Material and methods: The study involved 28 cardiologists and 84 internists who, based on a set of questionnaires, expressed their opinion on ambulatory treatment of patients with arterial hypertension. Also 21 internists, 6 cardiologists and 6 obstetrician-gynecologists expressed their opinion on treatment of pregnancy-induced hypertension.

Results: Cardiologists and internists recommended angiotensin-converting-enzyme inhibitors (ACE inhibitors) in 28% of cases, diuretics – in 23% of cases, beta-blockers (BB) – in 20, 4% of cases, calcium channel blockers (CCB) – in 13.7% of cases, angiotensin receptor blockers (ARB) – in 13.6% of cases for ambulatory treatment of patients with arterial hypertension. The antihypertensive drugs from other pharmacological groups (with central action, alpha-blockers, arteriodilators, sympatholytics, etc.) were prescribed in only 1.3% of patients. On treatment of hypertension in pregnancy showed that all physicians – 100% recommended as first-line agent for treatment of hypertension during pregnancy centrally acting antihypertensive drug Methyldopa. For second-line treatment they recommended CCB – in 36.36% of cases, alpha-adrenoblockers – in 24.24% of cases, BB – in 21.21% of cases, diuretics – in 12.12% of cases and 3% for ACE inhibitors and ARB. For treatment pre-eclampsia and eclampsia seizures in 84.85% of cases is recommended Magnesium sulfate and 15.15% mention labetalol, hydralazine and sodium nitroprusside.

Conclusions: ACE inhibitors, diuretics, BAB, CCB and ARB have been first-line drugs in the arterial hypertension (AHT) treatment. For treatment of pregnancy-induced hypertension physicians recommended centrally acting antihypertensive drug Methyldopa. For second-line treatment they recommended calcium channel blockers (CCB), alpha-adrenoblockers, beta-blockers (BB) and diuretics. As third-choice in treatment of severe hypertension in preeclampsia are selected direct vasodilators as hydralazine, labetalol, sodium nitroprusside, and the most widely used in preeclampsia and eclampsia remains magnesium sulfate.

Key words: arterial hypertension, antihypertensive drugs, angiotensin-converting-enzyme inhibitors

Introduction

The rational use of medicines remains one of the most important directions of public health, especially in socio-medical diseases, including arterial hypertension (AHT). Hypertensive disorders complicate 5-10% of the pregnant and still now remain a leading cause of maternal mortality. The medicine data based on available and currently known evidences in the field of clinical pharmacology allow determination of the principles of selection and assessment of the use of medicines in the above-named diseases, which allow not only the optimization of rational use but also the reduction of the necessary expenditures and the increase of the harmlessness of the treatment [3, 4, 16, 19].

The aim of the study was to perform a pharmaco-therapeutic analysis compared with the elucidation of the groups of drugs and antihypertensive medicines used in the treatment of patients with hypertension and treatment of hypertension during pregnancy [2,3,4,5,8,10,18].

Material and methods

A survey study was conducted out by physicians from the medical institutions of Chisinau municipality in order to assess the incidence of prescribing different groups of antihypertensive drugs in the period October, 2015 – November, 2017. The study involved 28 cardiologists and 84 internists who, based on a set of questionnaires, expressed their opinion on ambulatory treatment of patients with arterial hypertension to determine the following aspects: frequency of use of the main groups of antihypertensive drugs and their representatives in patients with hypertension; correspondence of the pharmacotherapy stage and the degree of hypertension; the share of fixed combinations drugs for complex pharmacotherapy of arterial hypertension. Also 21 internists, 6 cardiologists and 6 obstetrician-gynecologists expressed their opinion on treatment of pregnancy-induced hypertension to determine the frequency of use of main groups of antihypertensive drugs.

Results

The results of the carried out study showed that cardiologists and internists recommended angiotensin-converting-enzyme inhibitors (ACE inhibitors) in 28% of cases, diuretics – in 23% of cases, beta-blockers (BB) – in 20.4% of cases, calcium channel blockers (CCB) – in 13.7% of cases,

angiotensin receptor blockers (ARB) – in 13.6% of cases for ambulatory treatment of patients with hypertension. The hypotensive drugs from other pharmacological groups (with central action, alpha-blockers, arteriodilator, sympatholytics, etc.) were prescribed in only 1.3% of patients. The reported data are in accordance with the national and international guidelines recommendations and reflect the contemporary strategy of approaching the hypertensive patient in terms of the initial and final goal of antihypertensive therapy [2, 3, 4, 5]. The analysis of literature data reveals that ACE inhibitors, ARB, BAB, CCB and diuretics are considered first-line antihypertensive drugs in the AHT treatment, and alpha-adrenoblocks, central-acting drugs, vasodilators and renin inhibitors are part of second-line antihypertensive medicines [3, 4, 15, 28, 31, 33].

The analysis of the drugs prescription from each pharmacological group was relevant to the fact that doctors recommended a wide range of representatives, selection being based on the particularities of drug release (free or compensated), age aspects, associated diseases, and target organ damage. Among the ACE inhibitors, doctors mentioned in the questionnaires almost all medicines registered in the Republic of Moldova.

Thus, the most frequent drugs in this group were ramipril (28.9%) and lisinopril (28.5%), followed by captopril (18.9%), enalapril (18.2%) and perindopril (4.9%). The angiotensin-converting-enzyme inhibitors, as first-line drugs in the AHT treatment, have an overwhelming evidence base in numerous trials and clinical guidelines, including patients with associated diseases such as diabetes mellitus, ischemic heart disease, cardiac failure, cardiac arrhythmias, atherosclerosis, renal diseases [2, 3, 4, 6, 15, 22, 26].

The ACE inhibitors pharmacodynamic profile is quite varied in terms of blood levels changes of angiotensin-converting-enzyme, angiotensin I, angiotensin II, renin, aldosterone and haemodynamic parameters, and also by organoprotective properties. The mechanism of action particularities determine the decrease of angiotensin II levels by blocking the respective more significant enzyme in organs and tissues than in plasma, especially in long-term treatment. Concurrently, ACE inhibitors also inhibit kinase II by increasing bradykinin content by stimulating the release of nitrogen monoxide and vasodilator prostaglandins.

The hypotensive action of ACE inhibitors is also due to other mechanisms: sympathetic tone activity reduction (decrease in adrenaline and noradrenaline level); aldosterone secretion decrease (natriuretic effect); endothelin secretion suppression; endothelial dysfunction amelioration.

Hemodynamic (reduction of peripheral vascular resistance, post- and preload, increased renal flow, etc.) and cardiac effects (regression of left ventricular hypertrophy, increase of coronary flow, reduction of postinfarction mortality, improvement of cardiac insufficiency, decrease of nitrate tolerance) are responsible for diminishing the myocardial remodeling progression and vascular smooth muscle hypertrophy to prevent damage to organs and systems which reflect beneficially on the quality of life, morbidity and mortality of patients with cardiovascular diseases [3, 4, 6, 15, 16, 24, 26, 28].

Diuretics, as expected, occupied the second position in doctors' preferences. Indapamide (35.7%), spironolactone (26.8%), furosemide (16.5%), torasemide (12.7%) and hydrochlorothiazide (8.3%) were among the diuretics recommended to the patients. The use of diuretics in the HTA treatment is determined by the drugs ability to reduce blood pressure and to remove refractory mechanisms in other hypotensive groups. The antihypertensive action of diuretics is determined by various mechanisms depending on the duration of use. Thus, blood pressure lowering is achieved by the moderate effect of natriuresis with the reduction of circulating blood volume and preload, respectively, at the initiation of treatment (the first 4-6 weeks). The circulating blood volume is restored in long-term use and blood pressure lowering is possibly achieved by reducing vascular tone through several mechanisms: synthesis of vasodilator prostaglandins (indapamide, furosemide, torasemide); blockage of calcium channels (indapamide); activation of potassium channels (indapamide); reducing vascular intimal infiltration with sodium ions [3, 4, 20, 31, 33].

The quite frequent use of indapamide, a third generation non-thiazide diuretic, is argued by pharmacodynamic peculiarities, good tolerability and harmlessness, a salutary preparation in the long-term treatment of AHT. Indapamide, in addition to the hypotensive effect particularities, is characterized by beneficial influences on thiazides, on glucose metabolism (it does not produce hyperglycemia, does not disturb the sensitivity of peripheral tissues to insulin), lipid (a minimal effect on cholesterol level, triglycerides, increases the content of high density lipoproteins), electrolytic (practically does not produce hypokalaemia) and purine (does not increase uric acid level) [20, 31, 33].

The elucidation of new pathogenetic aspects of AHT, including refractoriness in the first-line drugs, has demonstrated the role of aldosterone in cardiovascular pathology through genomic and non-genomic mechanisms. Thus, the "aldosterone rickets" phenomenon was described, which occurs in AHT patients treated with inhibitors of the renin-angiotensin-aldosterone system due to incomplete inhibition of mineralocorticoid activity. The use of antagonist competitors of aldosterone (spironolactone, eplerenone), considered to be the second, third or even the forth-line drugs, under these conditions, contributed to the increase of efficacy and removing refractoriness in antihypertensive drugs [27, 30].

Spironolactone, a non-competitive antagonist of aldosterone, contributed to prevent progression of target organ damage and the development of complications, loss of potassium and magnesium ions. However, the high adverse reactions incidence (breast tightening, gynecomastia, erectile dysfunction, amenorrhea, hirsutism, etc.), determined by the steroid structure and influence on androgen receptors, limits patients' compliance with the treatment.

In this context, a particular interest is given to eplerenone, a selective antagonist of aldosterone, a product that is characterized by better tolerability and harmlessness. At the same time, it is estimated that, if spironolactone predominantly influences the mineralocorticoid genomic mechanisms, eplerenone is able to annihilate non-genomic ones, thus exhibiting a faster effect [1, 20, 27, 30, 31, 33].

The lower incidence of loop diuretics use is explicable because these drugs are prescribed by doctors in emergency situations, in severe AHT and associated with complications; in case of the renal function alteration (2^{nd}-3^{rd} degree). The rather high frequency of torasemide prescription due to its advantages over furosemide (effect up to 24 hours, less incidence of side effects, including the severe ones, better treatment compliance in patients) is significant [20, 31, 33].

Hydrochlorothiazide, although had the lowest use incidence, remains a thiazide in demand, especially in the forms combined with ACE inhibitors, ARB, BB, CCB, due to the moderate and constant antihypertensive effect, the ability to decrease the counterregulatory mechanisms in the case of refractoriness to other hypotensives, despite characteristic metabolic reactions [17, 20, 31, 33].

Beta-blockers were recommended relatively quite frequently (20.4%), which corresponds to the literature data [7, 8, 11, 19, 24]. Bisoprolol (45.7%), followed by metoprolol (29.2%), nebivolol (8.1%), and atenolol (7.7%) were most frequently prescribed drugs from this group. Carvedilol and propranolol were recommended with lower incidence. The relatively high incidence of BB in hypertensive patients is determined by an increase in the number of drugs of this group, especially cardioselective ones (bisoprolol, atenolol, metoprolol, acebutolol, etc.) and with vasodilatory action (nebivolol, carvedilol). The antihypertensive effect of BB is determined by several mechanisms: heart rate decrease; decrease of renin secretion and renin-angiotensin-aldosterone system activity; modification of the aorta baroreceptors sensitivity and sino-carotid glomerus; reduction of sympathetic central genesis; dilation of vessels by nitrogen monoxide production, alpha-adrenoreceptors blockade or direct myotropic action [3, 4, 7, 8, 29].

Among the CCB, the largest share goes to the dihydropyridine derivatives (73.1%), followed by the benzothiazepine derivatives (13.4%) and phenylalkylamine derivatives (10.7%). Dihydropyridines were represented by amlodipine (61.8%) and nifidipine (11.3%), and by lercanidipine, lacidipine, etc. in a smaller percentage.

Among the CCB with concomitant action on vessels and heart, diltiazem was prescribed in 13.4% of cases and verapamil in 10.7% of cases. The more frequent use of amlodipine is argued by pharmacological peculiarities: high bioavailability due to lipophilic properties; rapid onset of action; absence of neuroendocrine and sympathetic reflex mechanisms on heart and metabolism; the long half-life that determines convenience in administration (once a day) and patient's good compliance with the treatment; a lower incidence of side effects; increase in nitrogen monoxide production; the antioxidant properties presence.

These priorities determine the use of amlodipine as a first-line antihypertensive drug as a monotherapy and an important component in combined therapy with almost all hypotensive groups including patients with comorbidities (angina pectoris, atherosclerosis, diabetes mellitus, kidney disease) [3, 4, 11, 14, 23].

On the basis of randomized studies and meta-analysis, it was determined that CCB does not cede to ARB, ACE inhibitors, BAB, diuretics and alpha-adrenoblockers in blood pressure control, risk of cardiovascular (death, myocardial infarction, heart failure) and cerebrovascular events [14, 24].

Doctors recommended ARB with a similar incidence of CCB – 13,6%. Losartan (61.8%) and valsartan (33.2%) predominate among the drugs of this group. It is necessary to mention that in the doctors' preferences there were also mentioned such ARB as irbesartan (2.5%), telmisartan (1.25%), and candesartan (1.25%), drugs with some more advantageous pharmacological features welcomed in certain clinical situations. Angiotensin receptor blockers in European countries are prescribed to 20-25% of AHT patients because they are considered as the basic pathogenetic therapy that allows safe and adequate blood pressure control, prevention of target organ damage and complications. The use of ARB provides for a more complete blockade of the renin-angiotensin-aldosterone system because it blocks the action of angiotensin II on specific (type 1) receptors produced not only by angiotensin converting enzyme but also by alternative routes (chymase, etc.). At the same time, angiotensin II activity is maintained on 2nd type of angiotensin receptors while maintaining physiological effects (vasodilation, antiproliferative action, etc.). Concomitantly, metabolism of bradykinin, encephalins and other biologically active peptides, responsible for some specific side effects of ACE inhibitors that activate the quinine system, is not affected. The drugs of this group are characterized by a stable antihypertensive effect over 24 hours with a stable clinical effect after 3-4 weeks. Sartans exhibit favorable metabolic actions on lipid and carbohydrate metabolism, beneficial effects in patients with diabetes mellitus, dyslipidemias, metabolic syndrome, atherosclerosis of vessels. Clinical studies have shown decrease of glucose and insulin resistance level (stimulation of PPAR- nuclear receptors in adipose and muscle tissue, hepatocytes). It was proved the ARB capacity to reduce the contents of triglyceride and low density lipoprotein cholesterol. These effects, along with the improvement of endothelial dysfunction, will be welcomed in patients with the mentioned pathologies [3, 4, 6, 16, 22, 25, 26, 32].

Among the hypotensive preparations in other groups, the doctors have opted for centrally acting anti-hypertensives and alpha-1-adrenoblocking agents. It is necessary to mention that the imidazoline receptor agonist, moxonidine (52.5%), predominates among the medically active drugs compared to the methyldopa (20%) and clonidine (17.5%)

alpha-2 adrenomimetics. Among the selective alpha-adrenergic blockers, doxazosin was recommended in 10% of cases.

The therapeutic behaviour according to the degree of arterial hypertension was another aspect of the pharmacoepidemiological study. Thus, the investigations analysis revealed that doctors preferred monotherapy (86.4%), and the association of 2 drugs in 12.8% and association of 3 drugs only in 0.8%, in case of hypertension of the first degree.

In patients with arterial hypertension of second degree, the doctors mentioned the need for prescribing 2 drugs (65.5%), while monotherapy was sufficient in 21.2%, and triple therapy was required in 13.3% of patients. For hypertension of third degree, treatment with 3 hypotensive drugs (46.6%) is recommended. At the same time, doctors mentioned that about 38.7% of the patients responded adequately to treatment with 2 antihypertensives, and even 4 hypotensive preparations were recommended in 14.7% of patients. These data fully reveal the actual clinical situation due to target organ damage with the progression of high blood pressure.

The survey showed that doctors preferred associations between one drug from different groups (64.4%) in case of combined antihypertensive drug therapy, while fixed-dose combinations were recommended in only 26.7% of cases. The association of one drug with fixed-dose drugs combinations was used in 8.9% of cases.

The results on treatment of hypertension in pregnancy showed that all physicians -100% (21 internists, 6 cardiologists and 6 obstetrician-gynecologists) recommended as first-line agent for treatment of pregnancy-induced hypertension, centrally acting antihypertensive drug methyldopa. Accordingly to literature data methyldopa is one of the most widely used drugs for the treatment of hypertension during pregnancy (Category B, safe, accordingly to Food and Drugs Administration, FDA, USA classification of drugs in pregnancy). It is a prodrug metabolized to alpha methylnorepinephrine, which then replaces norepinephrine in the neurosecretory vesicles of adrenergic nerve terminals. Methyldopa inhibits vasoconstriction via a central mechanism by reducing catecholamine release [3, 4, 18]. It decreases central sympathetic outflow, decreasing systemic vascular resistance, without decreasing cardiac output BP control is gradual, over six to eight hours, because of the indirect mechanism of action. Treatment with methyldopa has been reported to prevent subsequent progression to severe hypertension in pregnancy and does not seem to have adverse effects on utero-placental or fetal hemodynamics. Adverse effects are based on pharmacodynamics effects – central alpha-2 blocking effect or decreased peripheral sympathetic tone. This drug can cause decreased mental alertness and impaired sleep, leading to sense of fatigue in some or depression in others. Still other observed side effects are decreased salivation, leading to xerostomia (chronic dry mouth), elevated liver enzymes in 5%; hepatitis and hepatic necrosis have been reported. Some patients will develop a positive antinuclear antigen or antiglobulin (Coombs') test with chronic use, which may occasionally cause clinical hemolytic anemia. However, it is not considered to be teratogenic based on limited clinical study data and forty-year clinical experience [3, 4, 8, 18]. Another central acting agent is clonidine (Category C, less safe, accordingly to FDA classification of drugs in pregnancy), a selective alpha-2 agonist, acts similarly and is comparable to methyldopa with respect to efficacy, but regarding to some safety concern there is a small controlled follow-up study with neonates that reported an excess of sleep disturbance in clonidine-exposed infants. So, in pregnancy, it is recommended to be used as a third-line agent for multidrug control of refractory hypertension [10, 18].

For second-line treatment our doctors recommended calcium channel blockers (CCB) – in 36.36% of cases, alpha-adrenoblockers – in 24.24% of cases, beta-blockers (BB) – in 21.214% of cases, diuretics – in 12.12% of cases and 3% for angiotensin-converting-enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers (ARB).

Calcium channel blockers have been used to treat chronic hypertension, mild pre-eclampsia presenting late in gestation and urgent hypertension associated with pre-eclampsia (Category C, less safe, accordingly to FDA classification of drugs in pregnancy, but amlodipine is classified as Category D-Teratogenicity in animals). Both nifedipine and verapamil are not associated with teratogenic risks to fetus exposed in first trimester. Maternal adverse effects with nifedipine include pharmacodinamic type as tachycardia, palpitations, peripheral edema, headaches, and facial flushing. Nifedipine does not seem to cause a detectable decrease in uterine blood flow. Short-acting dihydropyridine calcium antagonists sublingually are associated with maternal hypotension and fetal distress and are generally not recommended. In contrast long-acting oral nifedipine in pregnant patients with severe hypertension during pregnancy has been shown to be safe and effective. Dihydropyridine compounds: I-generation (nifedipine); II generation (felodipine, isradipine, nicardipina) also have a tocolytic effect and can delay the onset or slow the progression of labor. Phenylalkylamine agent as verapamil and benzothiazepine class as diltiazem may have additional value in women with proteinuria because of their antiproteinuric action [3, 4, 10, 18].

Both groups alpha-adrenoblockers and beta-blockers (BB) are recommended by our doctors with the same frequency (pindolol and acebutolol are classified as Category B, but atenolol, propronalol, metoprolol, timolol and labetalol – Categoty C). Accordingly to Cochrane analysis, beta-blockers were found to be more effective in lowering blood pressure than methyldopa. Labetalol, a nonselective alpha-and beta-blocker has obtained wide acceptance to treat sever hypertension in pregnancy, because of lower incidence of side effects in comparison with hydralazine- direct vasodilator Category C, associated with more maternal and perinatal adverse events, than other agents when used acutely [10,18].

Diuretics were recommended in 12.12% of cases, hydrochlorthiazide (Category B, safe), accordingly to FDA classification of drugs in pregnancy). Administration of diuretics in pregnancy remains a matter of dispute because of fluid and electolytes disturbances. The potassium sparing diuretics spironolactone, triamterene and amilorid are not recommended in pregnancy – Category D (teratogenicity in animals, accordingly to FDA classification of drugs in pregnancy).

Angiotensin-converting-enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers (ARB) were recommended in 3% of cases. These groups of antihypertensive agents that are used as first-line choice in treatment of essential hypertension are contraindicated in pregnancy because of severe toxicity secondary to reduced renal perfusion of the fetal kidneys (Category D, teratogenicity in animals, accordingly to FDA classification of drugs in pregnancy). Their use has been associated with renal dysgenesis, oligohydramnios as consequence of fetal oliguria, pulmonary hypoplasia, intrauterine growth restriction, and neonatal anuric renal failure, leading to death of the fetus. ARBs have also been associated with fetal demise and same concerns are applicable to the use of direct renin inhibitors. First trimester exposure to these agents has been associated with greater incidence of cardiovascular and central nervous system malformations. Whether these effects are secondary to hemodynamic effects or specific requirement of angiotensin II as a fetal growth factor is unknown. Patients should, therefore, be counseled to stop these medications while attempting to conceive. The risk of birth defects increased from 3 to 7% while on these medications at the time of conception [8, 10, 12, 13,18].

For treatment pre-eclampsia and eclampsia seizures in 84.85% of cases doctors recommended magnesium sulfate (Category A, most safe, accordingly to FDA classification of drugs in pregnancy).

Conclusions

On the basis of carried out study, it was found that ACE inhibitors, diuretics, BAB, CCB and ARB have been first-line drugs in the AHT treatment. The drugs selection was performed on the basis of national and international guidelines and the pharmacological properties of the antihypertensive drug groups. The use of second-line antihypertensive drugs (central-acting alpha-2-adrenomimetics, imidazolinone derivatives, alpha-1 adrenoblockers) denotes that the choice of antihypertensive therapy also takes into account the particularities of AHT evolution in the patient, especially in detected associated diseases and metabolic disturbances.

Based on the results obtained from the questionnaire on the treatment of pregnancy-induced hypertension, it was demonstrated that first-line agents remain central acting agent, alpha 2 adrenoreceptor agonist like methyldopa, which is in conformity with national and international protocols and guidelines. As second-line choice in the treatment of pregnancy-induced hypertension we can mention calcium channel blockers (CCB), alpha-adrenoblockers, beta-blockers and diuretics. As third-choice in treatment of sever hypertension in preeclampsia are selected direct vasodilators as hydralazine, labetalol, sodium nitroprusside and the most widely used in preeclampsia and eclampsia remains magnesium sulfate. It is very important to note that angiotensin-converting-enzyme inhibitors (ACE inhibitors) and angiotensin receptor blockers are contraindicated in pregnancy and must not be recommended for pregnancy-induced hypertension.

References

1. Caraus A, Bitca A. Antagonistii aldosteronului – perspective noi in tratamentul hipertensiunii arteriale severe [Antagonists of aldosterone - new perspectives in the treatment of severe arterial hypertension]. Buletinul Academiei de Stiinte a Moldovei [Bulletin of the Academy of Sciences of Moldova]. 2010;2(25):35-41. Romanian.
2. Ghidul ESH/ESC Managementul hipertensiunii arteriale 2013 [ESH/ESC Guide. Management of arterial hypertension]. Romanian Journal of Cardiology. 2013;23 Suppl C:1-66. Romanian.
3. Ghicavii V, Bacinschi N, Gusuila Gh. Farmacologie [Pharmacology]. 3rd ed. Chisinau; 2012. 996 p. Romanian.
4. Ghicavii V, Bacinschi N, Bumacov L, et al. Farmacologie clinică [Clinical Pharmacology]. Chisinau: Medicina; 2009. 1068 p. Romanian.
5. Ministerul Sanatatii al Republicii Moldova [Ministry of Health of the Republic of Moldova]. Hipertensiunea arterială la adult: Protocol clinic național [Arterial Hypertension in adult: National clinical protocol]. Vataman E, Curocichin G, Grosu A, et al., editors. Chisinau: [The Ministry]; 2014. 60 p. (PCN-1). Romanian.
6. Tantu MM. Antagonizarea farmacologică a sistemului renină-angiotensina-aldeosteron [Pharmacologic antagonism of the renin-angiotensin-aldeosteron system]. Acta medica transilvanica. 2012;22:89-92. Romanian.
7. Akbar S, et al. The current status of beta blockers' use in the management of hypertension. Saudi Med J. 2014;35(11):1307-17.
8. Brown CM, Garovic VD. Drug treatment of hypertension in pregnancy. Drugs. 2014 Mar;74(3):283-96.
9. Cruickshank JM. The role of beta-blockers in the treatment of hypertension. Adv Exp Med Biol. 2017;956:149-66.
10. Easterling TR. Pharmacological management of hypertension in pregnancy. Semin Perinatol. 2014 Dec;38(8):487-95.
11. Fares H, DiNicolantonio JJ, O'Keefe JH, Lavie CJ. Amlodipine in hypertension: a first-line agent with efficacy for improving blood pressure and patient outcomes. Open Heart. 2016 Sep 28;3(2):e000473.
12. Fitton CA, Steiner MFC, Aucott L, et al. In-utero exposure to antihypertensive medication and neonatal and child health outcomes: a systematic review. J Hypertens. 2017 Nov;35(11):2123-37.
13. Gillon TE, Pels A, von Dadelszen P, et al. Hypertensive disorders of pregnancy: a systematic review of international clinical practice guidelines. PLoS One. 2014;9(12):e113715.
14. Jeffers BW, Robbins J, Bhambri R. Efficacy of calcium channel blockers versus other classes of antihypertensive medication in the treatment of hypertensive patients with previous stroke and/or coronary artery disease: a systematic review and meta-analysis. Am J Ther. 2017 Jan/Feb;24(1):e68-e80.
15. Laurent S. Antihypertensive drugs. Pharmacol Res. 2017 Oct;124:116-125.
16. Li EC, Heran BS, Wright JM. Angiotensin converting enzyme (ACE) inhibitors versus angiotensin receptor blockers for primary hypertension. Cochrane Database Syst Rev. 2014 Aug 22;(8):CD009096.
17. Musini VM, Nazer M, Bassett K, Wright JM. Blood pressure-lowering efficacy of monotherapy with thiazide diuretics for primary hypertension. Cochrane Database Syst Rev. 2014 May 29;(5):CD003824.

18. Mustafa R, Ahmed S, Gupta A, et al. A comprehensive review of hypertension in pregnancy. J Pregnancy. 2012;105918
19. Park C, Wang G, Durthaler JM, Fang J. Cost-effectiveness analyses of antihypertensive medicines: a systematic review. Am J Prev Med. 2017 Dec;53(6S2):S131-S142.
20. Roush GC, Sica DA. Diuretics for hypertension: a review and update. Am J Hypertens. 2016 Oct;29(10):1130-7.
21. Savoia C, Volpe M, Grassi G, et al. Personalized medicine – a modern approach for the diagnosis and management of hypertension. Clin Sci (Lond). 2017 Nov 6;131(22):2671-85.
22. Shaikh A. A practical approach to hypertension management in diabetes. Diabetes Ther. 2017 Oct;8(5):981-9.
23. Tamargo J, Ruilope LM. Investigational calcium channel blockers for the treatment of hypertension. Expert Opin Investig Drugs. 2016 Nov;25(11):1295-1309.
24. Thomopoulos C, Parati G, Zanchetti A. Effects of blood pressure lowering on outcome incidence in hypertension: 4. Effects of various classes of antihypertensive drugs-overview and meta-analyses. J Hypertens. 2015 Feb;33(2):195-211.
25. Trimarco B, Santoro C, Pepe M, Galderisi M. The benefit of angiotensin AT1 receptor blockers for early treatment of hypertensive patients. Intern Emerg Med. 2017 Dec;12(8):1093-9.
26. Xue H, Lu Z, Tang WL, et al. First-line drugs inhibiting the renin angiotensin system versus other first-line antihypertensive drug classes for hypertension. Cochrane Database Syst Rev. 2015 Jan 11;1:CD008170.
27. Yugar-Toledo JC, Modolo R, de Faria AP, et al. Managing resistant hypertension: focus on mineralocorticoid-receptor antagonists. Vasc Health Risk Manag. 2017;13:403-11.
28. Weber MA, Schiffrin EL, White WB, et al. Clinical practice guidelines for the management of hypertension in the community: a statement by the American Society of Hypertension and the International Society of Hypertension. J Clin Hypertens (Greenwich). 2014 Jan;16(1):14-26.
29. Wiysonge CS, Bradley HA, Volmink J, et al. Beta-blockers for hypertension. Cochrane Database Syst Rev. 2017 Jan 20;(1):CD002003.
30. Belovol AN, Kneaz'kova II. Antagonisty aldosteronovykh retseptorov: klinicheskaia farmakologiia i terapevticheskaia effektivnost' pri khronicheskoi serdechnoi nedostatochnosti [Antagonists of aldosterone receptors: clinical pharmacology and therapeutic efficacy in chronic heart failure]. Mir Meditsiny i Biologii [The World of Medicine and Biology]. 2012;(1(32)):13-9. Russian.
31. Emst ME, Moser M. Ispol'zovanie diuretikov u patsientov s arterial'noi gipertenziei [Use of diuretics in patients with arterial hypertension]. Hypertension (Ukraine). 2010;2(10). Translated in Russian by Savust'ianenko AV. Russian.
32. Poteshkina NG, Selivanova GB, Zhalsaraev TJ. Antagonisty retseptorov angiotenzina II v lechenii gipertenzii: perspektivy klinicheskogo primeneniia [Angiotensin II receptor antagonists in the treatment of hypertension: perspectives for clinical use]. Arterial'naia gipertenziia [Arterial hypertension]. 2013;19(2):117-124. Russian.
33. Fomin VV. Diuretiki kak antigipertenzivnye preparaty: deistvitel'no li neobkhodimy poiski luchshego v klasse? [Diuretics as antihypertensive drugs: is it really necessary to find the best in the class?]. Hypertension (Ukraine). 2011; 3(17). Russian.

DOI: 10.5281/zenodo.1186188
UDC: 615.273.074:546.72

Quantitative analysis of the macromolecular complex of iron (III) hydroxide with polymaltose in liquid dosage forms

Oprea Vasile[1], Chem PhD, Associate Professor; **Cheptanaru Constantin**[1], Chem PhD, Associate Professor; ***Nistorica Mihai**[4], Pharm D; **Giza Cristina**[2], Pharm St; **Valica Vladimir**[2,3], MD, Pharm PhD, Professor

[1]Department of General Chemistry, [2]Department of Pharmaceutical Chemistry and Toxicology
[3]Scientific Center for Drug Research, [4]Medicines and Medical Devices Agency
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova
*Corresponding author: nistoricamn@gmail.com. Received January 15, 2018; accepted February 22, 2018

Abstract

Background: It is fundamental to develop new analysis methods for proving the utmost quality of medicines containing iron compounds. This study aims to determine whether the quantity of Fe (III) in dosage forms taken for analysis is equivalent to the concentration specified by the manufacturer.
Materials and methods: The concentration of Fe (III) in dosage forms taken for the study was determined using the standard addition method at acidic and alkaline pH using the sulfosalicylic acid as reagent. The obtained data was analyzed mathematically and graphically. The iodometric method of titration was applied to verify the obtained results.
Results: According to mathematical data analysis, the concentration of Fe (III) in the Ferimax syrup was 45.00 ± 2.23 mg/5ml at acidic and 50.11 ± 0.5 mg/5ml at alkaline pH. Using the graphical method, the concentration of Fe (III) was 43.75 mg/5ml at acidic and 50.60 mg/5ml at alkaline pH. For Ferrum Lek, the Fe (III) concentration was 50.68 ± 0.73 mg/5ml and 50.60 mg/5ml at alkaline pH using the mathematical and graphical methods. The redox titration method showed that in the Ferimax syrup, the average mass of Fe (III) is 44.07±0.7 mg/5ml, and in Ferrum Lek – 50.36±0.26 mg/5ml.
Conclusions: The concentration of Fe (III) in Ferimax syrup in acidic medium was lower than the reference of the label, while in basic medium both Ferimax and Ferrum Lek syrups have the expected concentration.

Key words: photometry, iodometry, iron polymaltose, Ferimax, Ferrum Lek.

Introduction

Iron plays an important role in the vital processes of the organism like oxygen and electrons transportation, DNA and steroids synthesis. It is an important structural part of hemoglobin, myoglobin, as well as the one of many enzymes. Because of its lack, symptoms like chronic fatigue, muscular weakness, loss of concentration, decreased resistance to stress and microbial infections or skin dryness can occur in the organism [1]. Among other medications, the doctors recommend the administration of drugs containing the complex of iron (III)-hydroxide with polymaltose for relieving the symptoms mentioned above [2]. According to the National Clinical Protocol "Iron deficiency anemia in children" approved by the Ministry of Health of the Republic of Moldova, decree No. 442 of 10.04.2013 among the medications used for the treatment of iron deficiency in children is listed the complex of iron (III) hydroxide with polymaltose in the following dosage forms: syrups, solutions or tablets [3].

The active ingredient of the above-mentioned dosage forms represents polynuclear molecules of iron (III) hydroxide surrounded by the polymaltose molecules thereby forming a stable complex. This way, the unionized iron does not interact with food and does not form reactive oxygen species which could damage the membranous structures of the gastro intestinal tract [4]. The structure of the complex maximally resembles that of the natural iron compounds with ferritin, a protein occurring naturally in the human body. Due to the similarity, the complex is absorbed through an active mechanism, and thus prevents the overdosage [5].

The advantage of iron polymaltose complex is a lower adverse reaction incidence in comparison with ionized Fe^{2+} compounds such as iron sulfate [6]. Thus, the development of new analyses methods for iron (III) is of importance. Its purpose is to ensure the good quality of the dosage forms. The aim of the study further exposed consists in the elaboration of new methods of quantitative analysis of iron (III) in two liquid dosage forms applying the photometric method of standard addition using the sulfosalicylic acid as the reagent.

Material and methods

In the Moldovan pharmacies, Ferimax and Ferrum Lek are the two most frequently found iron (III) containing syrups in doses equivalent to 50 mg/5ml of elemental iron. Thereby, both of these syrups were used in the study.

Laboratory glassware and instruments: volumetric flasks of different capacities, automatic pipettes from brand DAC-pette with volumes ranging between 100 – 1000 µl and 1000 – 5000 µl. In the titrimetric method the titrant volume measurement is a 2.0 ml microburette, while for photometric method the solution absorbance was determined by means of photoelectrocolorimeter KFK-2MP [КФК-2МП] at wavelengths of 400 and 490 nm using cuvettes of 1 cm

path length. The molecular absorption spectra were measured using the Agilent 5483 spectrophotometer. The experimental data obtained was statistically analyzed.

Standard solutions: Mohr Salt $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 6H_2O$ is the initial reagent used in photometric method which was recrystallized from distillated water. It was used for preparing the standard solution [7]. A probe of this salt with the mass equal to 0.17553 g was transferred to 500 ml volumetric flask and mixed with 50 ml of distillated water, 2.5 ml of 1 mol/l of H_2SO_4 and 0.5 ml of concentrated HNO_3 which was added for oxidizing Fe (II) to Fe (III). The solution was further heated up to the boiling point until its color changed to yellow. After cooling, the solution was diluted with distilled water till mark and was homogenized. The molar concentration of Fe (III) in the standard solution was $4.4762 \cdot 10^{-4}$ mol/l and the concentration of Fe (III) was 0.05 mg/ml.

Sulfosalicylic acid with the mass fraction of 10% was prepared using the following compound $C_6H_6O_6S \cdot 2H_2O$. The $\omega(NH_3)$ = 10% solution was prepared by diluting the 25% ammonium solution.

The iodometric method for oxidant titration was also used in the study for quantitative determination of Fe (III) in studied pharmaceutical forms, in which was used $Na_2S_2O_3$ solution. This solution was prepared from fixanal, using boiled purified water and cooled to room temperature.

The solution with $c(Na_2S_2O_3)$=0.01 mol/l was prepared by dilution also using boiled purified water and cooled to room temperature. The standardization was performed using $c(1/6KIO_3)$=0,01 mol/l standard solution which was prepared from a probe of weighed accurately KIO_3 (chemically pure). The ω(KI)=10% solution was made from a chemically pure probe of KI and the 0,5% starch solution was prepared as usual.

Sample preparation: A 50 ml volumetric flask plugged with a cap was preventively weighed. After adding 0.9 ml of syrup using an automated pipette the volumetric flask was weighed with its plug cap again and the mass of the syrup sample was determined by difference. After, 2-3 ml of distillated water and 2.0 ml of 1 mol/l of H_2SO_4 were added to the flask and it was placed in a 100°C heated water bath. The dark brown color of the sample solution disappeared after 1.5 – 2.0 minutes and changed to light yellow color for each syrup. After cooling the solutions were brought to volume with purified water, and homogenized. The sample solution of Ferrum Lek had a light-yellow tint and the one of Ferimax a light purple tone. Distillated water was added up to the graduation marking.

Fe (III) quantitative analysis procedure: The quantitative analysis of Fe (III) from the sample solutions was performed using the photometric method of standard addition having sulfosalicylic acid as the reagent [8,]. The same volume of the sample solution was added to a few 50 ml volumetric flasks. Starting the second volumetric flask, increasing quantities (ml) of standard Fe (III) solution and 4 ml of 10% sulfosalicylic acid were successively added. For creating a basic pH, 4.0 ml of 10 % ammonium solution was additionally mixed. In the end, distillated water was added up to 50 ml and the formed solution was left for 10 minutes before measuring the absorbance. Afterwards, their absorbances were measured at the respective wavelength.

The iron (III) in the slightly acidified solutions to be analyzed of the studied syrups was determined quantitatively also by iodometric method of oxidants dosage, according to the following procedure. In a titration beaker for each analysis different, but exactly measured volumes of syrup solution to be analyzed by means of automatic pipette was added. Later 5-6 ml of solution with ω (KI) = 10% was added and the solution obtained was left in rest for 5 minutes. After this, the iodine released in an amount equivalent to the Fe (III) content in the solution to be analyzed was titrated with standardized $Na_2S_2O_3$ solution. To the end of the titration in the titration beaker was added 6-8 drops of starch solution and the titration was continued until the blue color of the solution appeared.

Results and discussion

Sulfosalicylic acid is used for the quantitative determination of iron (III) at acidic pH (1.8 – 2.5) forming Fe (III) mono-sulfosalicylate which has a purple color (maximum absorption at λ_{max} = 510 nm [11], or at λ_{max} = 505 nm according to [12]).

At basic pH (9.0 – 11.5) a yellow colored complex compound is formed (λ_{max} = 416 nm [11], but according to [13] the absorbance is measured at λ = 420 – 430 nm) in which the ratio between the metal and the ligand is 1:2 [11]. Fe (II) and Fe (III) can be determined separately using the photometric method at this pH as well as their total amount when they are both present in the solution [10, 13].

Preventive experiments have demonstrated that the absorption spectrum of the Ferimax syrup solution and the spectrum of a standard solution of Fe (III) with sulfosalicylic acid are identical in both the acidic and the basic media and absorb maximum electromagnetic radiation at λ_{max}=504 nm and λ_{max}=424 nm (fig. 1).

Similar spectra to those from fig. 1 which absorb maximum electromagnetic radiation at the same wavelengths in the acidic and basic media were obtained using the Fe (III) solution obtained from Ferrum Lek syrup with sulfosalicylic acid.

In the photometric method for the quantitative analysis of Fe (III) in the solutions to be analyzed, obtained from the studied pharmaceutical forms, the standard additions method was used, which was used in two variants: the calculation method and the graphical method [8, 10]. The more detailed essence of the calculation method and the final formula used to calculate the results of the analysis was previously described [10, 14]. In this report we present only the final formula after which the iron mass (m_{Fe}, mg/5ml) in the liquid pharmaceutical form was calculated by the calculation method of the standard addition:

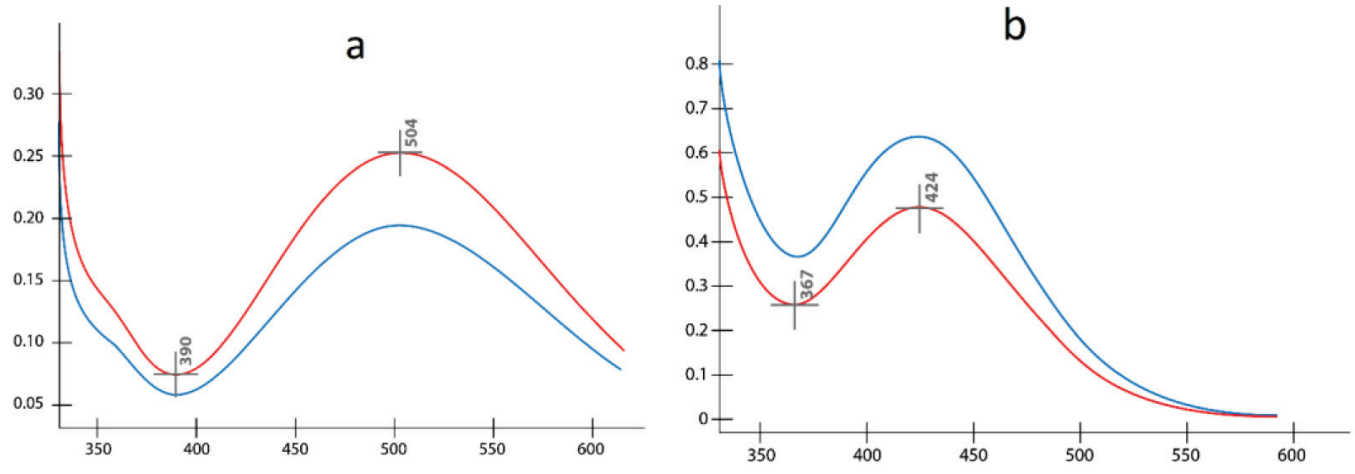


Fig. 1. Absorption spectra of the solution to be analyzed in: a – acidic medium, b – basic medium.

$$m_{Fe} = \frac{A_x \cdot m_i \cdot V_i \cdot V_0 \cdot \rho \cdot 5}{(A_{x+a} - A_x) \cdot V_1 \cdot m_p} \quad (1)$$

in which:

A_x – the absorbance of the solution, in the preparation of which a certain volume of the solution to be analyzed of respective solution with the unknown Fe (III) mass;

A_{x+a} – the absorbance of the solution, in the preparation of which a certain volume of the solution to be analyzed of respective solution with the unknown Fe (III) mass, but also with the addition of a certain volume of standard Fe (III) solution;

m_i – the iron content in the initial standard solution of Fe (III), mg/ml;

V_i – volume of initial standard solution of Fe (III), which was taken for preparation of the solution with the A_{x+a} absorbance, ml.

V_0 – the capacity of the volumetric flask with the initial solution to be analyzed after decomposition of the complex of iron (III)-hydroxide with polymaltose from respective liquid pharmaceutical form taken for analysis, ml;

V_1 – the fraction of the initial Fe (III) solution to be analyzed after decomposition of the complex of iron (III)-hydroxide with polymaltose from respective liquid pharmaceutical form taken for analysis, ml.

m_p – mass of the sample solution taken for analysis, g;

ρ – the density of the syrup, g/ml.

The data obtained from the experiment, as well as the Fe (III) mass calculated using equation (1) is presented in the tables 1a, 1b and 2.

Table 1

Data for determination of Fe (III) content with sulfosalicylic acid in the Ferimax syrup by standard addition method

a – acidic pH

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	1.0	-	-	0.105	-
2	1.0	1.0	0.05	0.139	42.89
3	1.0	2.0	0.10	0.172	43.53
4	1.0	3.0	0.15	0.202	45.10
5	1.0	4.0	0.20	0.229	47.04
6	1.0	5.0	0.25	0.262	46.45

(V_0= 50 ml; m_i=0,05 mg/ml; m_a=m_i·V_i; m_p=1,0572 g; ρ=1,1747 g/ml; pH 2,0 – 2,1)

b – basic pH

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	0.5	-	-	0.082	-
2	0.5	0.5	0.025	0.123	50.00
3	0.5	1.0	0.050	0.165	49.40
4	0.5	1.5	0.075	0.204	50.41
5	0.5	2.0	0.100	0.246	50.00
6	0.5	2.5	0.125	0.287	50.00
7	0.5	3.0	0.150	0.324	50.83

(V_0= 50 ml; m_i=0,05 mg/ml; m_a=m_i·V_i; m_p=0,58155g; ρ=1,1631 g/ml)

Table 2

The experimental data for determination of Fe (III) content with sulfosalicylic acid in the Ferrum Lek syrup by standard addition method in basic medium

No.	V_1, ml	V_i, ml	m_a, mg	A_x & A_{x+a}	m_{Fe}, mg/ml
1	0.5	-	-	0.085	-
2	0.5	0.5	0.025	0.127	50.60
3	0.5	1.0	0.050	0.171	49.42
4	0.5	1.5	0.075	0.211	50.60
5	0.5	2.0	0.100	0.251	51.20
6	0.5	2.5	0.125	0.294	50.84
7	0.5	3.0	0.150	0.333	51.41

(V_0= 50 ml; m_i=0.05 mg/ml; m_a=m_i·V_i; m_p=0.60165g; ρ=1.2033 g/ml)

Data from tables 1a, 1b, 2 and the average concentration of Fe (III) (mg/5ml) having a 0.95% confidence interval were statistically analyzed. The average concentration of Fe (III) (mg/5ml) in the Ferimax syrup equals to 45.00±2.23 at acidic pH and 50.11±0.5 at basic pH (tab. 1 a and 1 b). The average concentration of Fe (III) (mg/5ml) from the Ferrum Lek syrup at basic pH is 50.68±0.73 (tab. 2).

Data from tables 1a, 1b and 2 were used for photometric determination of Fe (III) concentration applying the graphical method of standard addition. For this, the functional dependence $A_{x+a} = f(m_a)$ was plotted, in which m_a is the mass of the addition. This dependence represents a straight line that intersects the A_x value on the Y – axis, which contains only solution to be analyzed with unknown mass of Fe (III). At the extension of these three straight lines to the intersection with the X – axis, the segments $-m_{a=}\ m_x$ were obtained [10, 14]. For Ferimax syrup in the acidic medium $\overline{m}\overline{m}_x = 0.1575$ mg was obtained, while in the basic medium for both syrups $\overline{m}\overline{m}_x = 0.0506$ mg was obtained. The mass of Fe (III) (mg/5ml) in the syrups was calculated using the following equation:

$$m_{Fe} = \frac{m_x \cdot V_0 \cdot \rho \cdot 5}{V_1 \cdot m_p} \quad (2)$$

in which all the notes see above

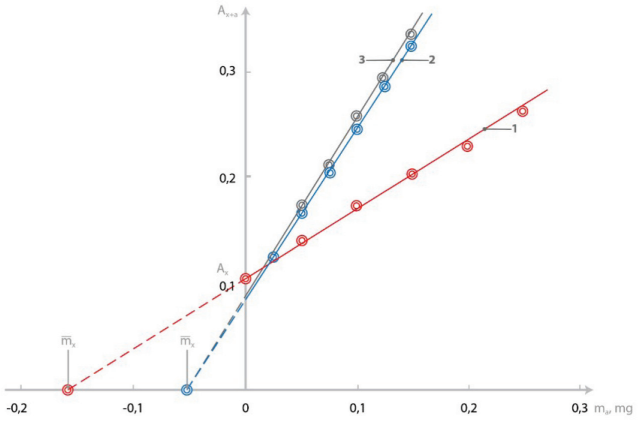


Fig. 2. The Ax+a = f (ma) dependence for photometric determination of Fe (III) with sulfosalicylic acid applying the graphical method of standard addition in:
1 – acidic medium (Ferimax), 2 – basic medium (Ferimax), 3 – basic medium (Ferrum Lek).

The data in tables 1a, 1b and 2 were used for the photometric determination of the Fe (III) mass in the solutions to be analyzed of studied syrups using equation 2 by the graphical method of the standard addition. For Ferimax syrup, the Fe (III) mass in the solution to be analyzed in the acidic medium constituted 43,75 mg/5ml and in basic media the Fe (III) mass in the solution to be analyzed in this syrup was the same as in the solution to be analyzed of Ferrum Lek syrup and constituted 50.60 mg/5ml.

The results obtained in the basic medium by the graphical method of the standard addition for the studied pharmaceutical forms fall within the limits of the acceptance of the obtained mass of Fe (III) in the solutions to be analyzed of these syrups by the photometric method of calculation in the same medium and practically correspond to the data of the manufacturing companies. However, in the basic medium with sulfosalicylic acid the photometric method determines both the Fe (II) and Fe (III), or the sum content of Fe (II) and Fe (III) in the analyzed solution [9, 10, 13]. At the same time, the quantitative determination of the Fe (III) mass in the Ferimax syrup in the acidic medium by the calculation and graphical photometric method of the standard addition showed lower results than those in the basic medium.

A different method of analysis was used to confirm the results obtained by the photometric method. This is the iodometric titration method proposed by the Chinese Pharmacopeia for the analysis of macromolecular complex compound of $Fe(OH)_3$ with polymaltose (iron dextran) [15].

The solutions taken for analysis were the same ones used in the photometric method. In contrast to the iodometric method described in the monograph [15], $KMnO_4$ was not added before adding KI solution. In addition, the diluted titrant solution was used to titrate the iodine eliminated in an amount equivalent to the Fe (III) content in the solution taken for the titration. The results obtained are presented in tables 3 and 4.

Table 3

Iodometric determination of Fe (III) in the Ferimax syrup

No.	V_1, ml	$V(Na_2S_2O_3)$, ml	m_{Fe}, mg/5ml
1	2.0	0.550	45.03
2	2.5	0.680	44.54
3	3.0	0.805	43.94
4	3.5	0.920	43.04
5	4.0	1.090	44.62
6	4.5	1.185	43.12
7	5.0	1.350	43.18

(K=1.0554; V_0= 50 ml; m_p=1.0572g; ρ=1.1747 g/ml)

Table 4

Iodometric determination of Fe (III) in the Ferrum Lek syrup

No.	V_1, ml	$V(Na_2S_2O_3)$, ml	m_{Fe}, mg/5ml
1	3.00	0.96	50.83
2	3.50	1.11	50.38
3	4.00	1.26	50.04
4	4.25	1.34	50.08
5	4.50	1.43	50.48
6	4.75	1.51	50.50
7	5.00	1.58	50.20

(K=1.0239; V_0= 50 ml; m_p=1.0898 g; ρ=1.2109 g/ml)

The mass of Fe (III) (m_{Fe}, mg/5ml) in the respective syrup was calculated using the following equation:

$$m_{Fe} = K \cdot V(Na_2S_2O_3) \cdot 0{,}5585 \cdot \frac{V_0 \cdot \rho \cdot 5}{V_1 \cdot m_p}, \quad (3)$$

K – correction coefficient of the titrant concentration in relation to the theoretical concentration of the 0,01 N $Na_2S_2O_3$ solution;

$V(Na_2S_2O_3)$ – volume of the equivalence of the titrant, ml;

0.5585 – Fe (III) mass equivalent to 1 ml of $Na_2S_2O_3$ (c= 0.01 mol/l), mg/ml.

For more details see equation (1).

The data from tables 3 and 4 was statistically processed to obtain the mean mass of Fe (III) (mg/5ml) determined based on a 95% certitude. For the Ferimax syrup, by titration method, an average Fe (III) mass value of 44.07±0.7 mg/5ml was obtained, and for Ferrum Lek – 50.36±0.26 mg/5ml.

Conclusions

If a compound contains ions of both Fe (II) and Fe (III), through quantitative analysis using sulfosalicylic acid at acidic pH, only Fe (III) can be determined. When Fe (II) and Fe (III) are both present in a solution, the total iron amount is analyzed at basic pH using the same physico-chemical method of analysis mentioned above [8, 9, 12]. The photocolorimetric standard addition method was applied in two variants: graphic and calculation methods as well as the iodometric titration method. Two syrups produced by two different manufacturers with brand names: Ferimax and Ferrum Lek were used for the study. The results obtained after statistical analysis showed that at acidic pH the concentration of Fe (III) in Ferimax is less than 50mg/5ml which proves the presence of Fe (II) ions. While analyzing Ferrum Lek at basic pH also using the statistical methods the Fe (III) concentration was determined to be 50mg/5ml which is the same as mentioned by the manufacturer. The results obtained via the photometric method were confirmed using the iodometric titration method of oxidants, which also proved that the concentration of Fe (III) is less than 50mg/5ml in the Ferimax syrup, while the Fe (III) content in the Ferrum Lek syrup does not have any deviations from the concentration specified by the manufacturing company.

References

1. FAO (Food and Agriculture Organization), WHO (World Health Organization). Human vitamin and mineral requirements: Report of a joint FAO/WHO expert consultation. Rome: FAO; 2002. Chapter 13, Iron; p. 195-221.
2. Jacobs P, Wood L, Bird AR. Erythrocytes: Better tolerance of iron polymaltose complex compared with ferrous sulphate in the treatment of anemia. Hematology. 2000;5(1):77-83.
3. Turea V, et al. [Iron deficiency anemia in children]. Chisinau: Ministry of Health of the Republic of Moldova; 2013. 25 p. (National Clinical Protocol; 111). Romanian.
4. Geisser P, Burckhardt S. The pharmacokinetics and pharmacodynamics of iron preparations. Pharmaceutics. 2011;3(1):12-33.
5. Reddy PS, Adsul BB, Gandewar K, et al. Evaluation of efficacy and safety of iron polymaltose complex and folic acid (Mumfer) vs iron formulation (ferrous fumarate) in female patients with anaemia. J Indian Med Assoc. 2001;99(3):154-65.
6. Chandrakala Kambar, Zahedabano, Meenakumari A. Comparative study of efficacy and safety of iron polymaltose complex with ferrous sulphate in antenatal women with moderate anemia. IOSR J Dent Med Sci. 2013;9(1):9-13.
7. Kariakin IuV, Angelov II. Chistye khimicheskie veshchestva [Pure chemicals]. Moscow: Khimiia; 1974. p. 340. Russian.
8. Bulatov MI, Kalinkin IP. Prakticheskoe rukovodstvo po fotometricheskim metodam analiza [Practical guide for photometric analysis methods]. 5th ed. Leningrad: Khimiia; 1986. p. 178., p. 432. Russian.
9. Oprea V, Cheptanaru C, Valica V. Analiza fotometrică a Fe în forme farmaceutice solide [Photometric analysis of Fe in solid pharmaceutical forms]. Revista farmaceutică a Moldovei [Moldovan pharmaceutical journal]. 2014;(3-4):53. Romanian.
10. Oprea V, Cheptanaru C, Valica V, Nistorica M, Remis V. Determinarea ferului cu acidul sulfosalicilic în comprimate de Feroplect prin metoda fotometrică a adaosului standard. [Iron analysis from Feroplect tablets using the photometric method of standard addition with sulfosalicylic acid]. Revista farmaceutică a Moldovei [Moldovan pharmaceutical journal]. 2015;(1-2):28-31. Romanian.
11. Dorokhova EN, Prokhorova GV, Analiticheskaia khimiia. Fiziko-khimicheskie metody analiza [Analitical chemistry. Physico-chemical analytical techniques]. Moscow: Vysshaia shkola; 1991. p. 231, 256. Russian.
12. Pozdnyakov I, Plyusnin V, et al. Photochemistry of Fe(III) and sulfosalicilic acid aquaeous solutions. J Photochem Photobiol A Chem. 2006;182:75-81.
13. Sharlo G. Metody analiticheskoi khimii. Kolichestvenyi analiz neorganicheskikh soedinenii [Analitical chemistry methods. Anorganic qualitative analysis]. Moscow: Khimiia; 1965. p. 976. Russian.
14. Oprea V, Cheptanaru C, Valica V, Nistorica M, Remis V. Photometric determination of Fe (III) with sulfosalicylic acid using the standard addition method in oral drops Ferropol. Curierul medical (Chisinau). 2016;59(2):11-4.
15. China Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China. English ed. (known as Chinese Pharmacopoeia). Beijing: China Medical Science Press; 2010. p. 650-654.

DOI: 10.5281/zenodo.1186194
UDC: 614.2/.4(478)

Medical-economic reasonings on the reform in the field of state surveillance of public health

Pantea Valeriu, MD, PhD, Associate Professor

The Scientific Laboratory of Management in Public Health, National Centre of Public Health
Chisinau, the Republic of Moldova

Corresponding author: valeriu.pantea@cnsp.md. Received January 11, 2018; accepted February 26, 2018

Abstract

Background: The functional organization of the service, institution or organization requires adjustment to challenges of the social, economic, and professional environment that intervene development stages of society. This is also true for the Public Health Supervision Service.
Material and methods: The results are based on evidence of historical achievements, statistical activity data in public health surveillance, the disease prevention, data on managers' opinion about ways to streamline institutional work, including some results of the economic activity of the institutions.
Results: The diminished activity in the prevention of non-communicable diseases is determined by the scarce budget for public health surveillance activities, the lack of staff and the inappropriate approach of the methodology for monitoring environmental factors in relation to the health of the population. Service overloading 1.2-1.4 times to the functional standard, driven by low wages and as a result of staff exodus, has a negative impact on effective indicators of public health coverage. The current model of non-communicable disease prophylaxis, with a particular focus on increasing healthcare investment, does not contribute to improving the population's health indicators.
Conclusions: The functioning of the Public Health Surveillance Service under the conditions of insufficient budget with dispersion of the existing number of institutions does not contribute to the efficiency of public health surveillance.
The results of the study, also the health and economic indicators in the health system point to the need for institutional reform to adjust the public health surveillance and financial support for disease prevention activities under the new conditions.

Key words: health surveillance, legislation, indicators, prophylaxis, efficiency, reform, budget.

Introduction

Activities of primary prophylaxis of diseases, protection and promotion of health are declared by law as basic fields in functioning of public health surveillance institutions of our country. In the world, every country defines its own "social institute" – or a system to achieve the mission of public health. Legislation in force of the Republic of Moldova sets the general requirements regarding the public health surveillance, the rights and obligations of individuals and organizations and the way of organizing the system in this field [1].

The foundation of a nationwide system, with tasks in prevention and prophylaxis of undesirable health events, comes out of the necessity to diminish the negative impact, the social, economical and medical burden that diseases impose on society.

The history of the development of the service of public health surveillance has its own stages of evolution, being a part of health system and the successor of the ex-Soviet sanitary-antiepidemic service, organized in the country in the far postwar years. The activity of the sanitary-antiepidemic, or sanitary-epidemiological service (later) in the soviet period (years 1944-1991) was governed by party decisions (The CPSU)[1)] and decisions of the Council of Ministers of the USSR[2)], that approved the operating rules of the service, through modifications and further adjustments – a mandatory act for all former union republics.

Thus, the development and efficiency of this system functioning, under such conditions (structures and institutions), were meant to be directly connected with population health, preventive measures undertaken, the level of science development, and last, but not the least, in relation to the economic capabilities of the state, provided for these purposes.

During this period (years 1944-2018) a series of stages followed, marked by certain achievements depending on the level of social– economic development of the state, such as:

- creation of primary sanitary-antiepidemic structures, including sanitary inspection, elimination of the consequences of war, prophylaxis and fight outbreaks of communicable diseases and epidemics, liquidation or reduction of the incidence of infectious diseases (years 1944-1956) [3)] [2,3];
- the integration of preventive and current health surveillance – as a fundamental principle in the functioning of the Service (1956-2016);
- the creation of a self-sustaining service within the

[1)] CPSU – The Communist Party of the Soviet Union
[2)] USSR – The Union of Soviet Socialist Republics

[3)] Establishing the first anti-epidemic organizational structures in the country: – Republican Station for the control of malaria,1944, – Republican sanitary-bacteriological laboratory, 1944, – Institute of scientific researches in epidemiology and microbiology, 1947, – Sanitary– epidemiologic Republican Station, 1948.

health system[4]), along with the development of its technical and material basis and the strengthening of the professional potential, the creation of the sanitary and hygiene faculty within Nicolae Testemitsanu State Univrersity of Medicine and Pharmacy;
- strengthening and developing the Service infrastructure, continuing the development and implementation of progressive forms of activity by: extending the sanitary and antiepidemic surveillance measures, improving the administration of the Service, (years 1968-1973);
- extending the powers of the Service representatives in health surveillance, including by regulating the application of administrative measures and sanitary-epidemiological restrictions (through prescriptions, prohibitions and / or stopping the exploitation of the objectives) (years 1974-1990);
- organizing the State Sanitary and Epidemiological Service of the Republic of Moldova (the period of independence of the country), the economic crisis with its consequences: in financing, in the gradual decrease of the units in the Service, the exodus of personnel, the evolution of the reformation of the contents and tasks of the state sanitary-epidemiologic service, evolving from sanitary-epidemiological stations to hygiene and epidemiology centers, to preventive medicine centers and to public health centers, years1991– 2009 [4];
- the adoption of the Law of the Republic of Moldova no. 10 of 2009 – with the subsequent organization of the State Public Health Supervision Service under conditions of community association of the country (years 2009-2016);
- finally, reforming the Service with the creation of the National Public Health Agency and centers of excellence in public health – optimizing the number of institutions and staff (year 2017-present) [5].

The period of domination, in the past, of infectious pathologies in the structure of morbidity and mortality, frequently, with epidemic character and the effect of "depopulation" of territories (by typhoid fever, cholera, dysentery, malaria, trachoma, exanthematic typhus, anthrax, etc.) determined medical science and practice to propose governments to establish certain forms and methods of organizing and fighting these epidemies and hazards. The knowledge of the particularities of the manifestation and spread of these health phenomena, has contributed to hygienic and epidemiologic argumentation of ways and forms of intervention for the localization, stopping, treating and eliminating epidemics, epidemic outbreaks and cases of diseases extremely dangerous for the society.

An important role of success in this fight was due to the complex approach of the antiepidemic measures and the prophylactic principle, implemented through the creation of certain structures and institutions, eventually – of the state functioning system in order to combat these phenomena.

The dissolution of the Soviet state in the 90s (XX century) and the achievement of the country's independence – a process accompanied by the dysfunction of the professional relations with the profile institutions of the former USSR, followed by the economic crisis [5], as well as the transition of the branches of the national economy to new economic relations, on the whole, dictated the need to adjust institutions to operate the system under the new conditions, including the public health sphere. Thus, the way of financing the sanitary-antiepidemic service, at that stage, according to the ex-Soviet model – only from the limited state budget, over the time, has caused gaps in adjusting the technical-material basis to the occurring events, including the lack of resources for the purchase of vaccines and provision of possibilities for diseases' diagnostics, of identification of new chemical substances in products and materials etc. [6,7]. Their negative result has not been long awaited, undesirable phenomena occurring in the country in the form of epidemic outbreaks and outbreaks of infectious diseases (of diphtheria, measles and rubella, mumps, cases of anthrax, increased number of cases of hepatitis and HIV/AIDS etc.). As a result, during this period, the main health indicators, manifested by high morbidity and mortality, including the infants, are significantly worsening, creating a considerable decline in demographic indices in society.

The created situation imperatively dictated the search for solutions for the recovery of the population's health. The only relevant solution, undertaken by the management of the State Sanitary-Epidemiological Service (SES), at that time, was to appeal to international institutions and bodies, to grant the Republic of Moldova [5]) a help in recovery of the situation. Thus, starting from year '93 (XX century) and up till now, the country was supported financially (and materially) through WHO[6]), USAID[7]), GAVI[8]), UNICEF[9]), SDC[10]), European Council and others, in the purchase of vaccines, in fortification of the technical-material basis of the Service, in the implementation of sanitation projects, but also in organizing population surveys (MICS2[11]), MICS4, STEPS[12]) and others). Subsequently, the results of these population surveys represented the main arguments and evidence in the development of National Programs, Regulations and Sanitary Standards, The National Public Health Strategy, etc.

[4] Until this stage, profile structures functioned within medical-sanitary institutions.

[5] Republic of Moldova became a member of the World Health Organization on 4th May 1992.

[6] WHO – World Health Organization;

[7] USAID – U.S. Agency for International Development;

[8] GAVI – Global Alliance for Vaccines and Immunisation

[9] UNICEF – United Nations International Children's Emergency Fund;

[10] SDC – The Swiss Agency for Development and Cooperation;

[11] MICS – Multiple Indicator Cluster Survey;

[12] STEPS – Study of the prevalence of risk factors for non-communicable diseases

Material and methods

The purpose of the study consisted in identification of the judgments on developing and institutional – functional adjusting of public health service to new conditions created in the sphere of health, including the period of community association of the country. The research is based on the complex analysis of the results of the activities of the institutions of the State Service of Public Health Surveillance (in the period 1995-2016) and the results of the opinion poll of the managers, on the aspects of institutional management. Complimentary research was based on the results of the human and material potential analysis of the Institutions in the Service. The opinion of managers (of institutions and structures) was studied on the basis of the questionnaire [13] consisting of 62 closed and open questions with 247 variables. The survey comprised 71 managers of level one and two (i.e. chief physicians and heads of departments from 35 territorial CPH), which accounted for 57.3% of the total number of managers (n = 124) of the Service. The lot consisted of respondents with great practical experience as managers; the share of respondents that had a 15 year experience in management represented 80%.

In the research we have used the methods: historical, epidemiological, statistical, questionnaire (survey) and economic. The study also used statistical databases of the National Bureau of Statistics (www.statistica.md) and the National Public Health Center (NPHC) www.cnsp.md.

Results and discussion

The activities carried out by the specialized institutions of the Public Health Surveillance Service, according to its tasks [1,8,11], during the period 1995-2016, had the expected effect, contributing to the stability of the epidemiological state concerning communicable diseases. As a result, communicable diseases in the general morbidity structure currently account for 11%, compared to year 1990, while the share of transmissible diseases was about 29.6%[14]).

The results of the analysis of the planned measures and those carried out during this period by the institutions, were mainly focused on prevention and prophylaxis of communicable diseases and less in the field of non-communicable disease prevention (tab. 1).

Thus, insufficient approach to non-communicable disease prevention methodology, along with managerial aspects, through which cross-sectoral health promotion activities are underdeveloped – all on the whole, does not contribute to improving health indices. Therefore, the conditions created continue to maintain the high rate of general morbidity (8118.8‰o.o) and mortality, including that of working age (about 50.6‰o.o) [9]. The process of gradual decrease of

[13] Questionnaire approved by the Scientific Council of NCPH and through the Provision No 667d of 01.11.2016 "On the organization of the survey on the assessment of managerial aspects in public health surveillance".

[14] Database, on infectious diseases, 1993-2016 // Form 2, NCPH (without pneumonia, acute bronchopneumonia).

Table 1

Share of preventive and prophylactic activities of communicable and non-communicable diseases in the activity of the SSSP Service institutions (years 2010-2016)

Indices	In the prevention and prophylaxis of communicable diseases	In the prevention and prophylaxis of non-communicable diseases
Share of planned activities by institutions, (in %)[15]	68.2	31.8
Share of activities carried out according to annual reports /statistical reports /, (in %)	80.9	19.1

[Source: made up by the author based on the analysis of planned activities in 36 territorial CPH and statistical reports]

communicable diseases incidence (due to specific and non-specific prophylaxis measures) and increasing prevalence of non-communicable diseases modified the structure of general morbidity, placing in the foreground the morbidity and mortality caused by non-communicable diseases (fig. 1).

In our opinion, there are several reasons for this situation, and the first one is caused by the "old working habits" of the Service's specialists, who are oriented towards prevention of communicable diseases, which implies a well–known methodology practiced for a long time, compared to the more complex methodology for preventing non-communicable diseases. The methodology of prevention of non-communicable diseases is carried out through a multidirectional spectrum of measures, with intervention in risk factors and determinants of health [10,11]; they are demanding major effort in undertaking of intersectoral and extended measures over time. Measures of preventing and prophylaxis of non-communicable diseases must be substantiated by evidence based on special research (of interrelation), where the values of the environmental factors must be co-reported in the time, space and specific population of the research area. Currently, the values of the factors of the external and internal environment [12], although managed, monitored (investigated, measured, evaluated) daily and/or monthly, their values are not reported in time to the medical centers about the state of health of the population in a specific environment.

Nowadays, research of the health state in relation to the factors that determine it, is carried out with deviation from the classical methodology, the reason, according to answers of respondents, is the lack of possibilities and institutional potential capable of carrying out a sampled research and complying with unanimously accepted regulations, according to the rules of health research outlined in the literature.

Actually in this situation, another reason cannot be

[15] Based on the assessment of the territorial PHC activity plans (years 2010-2016).

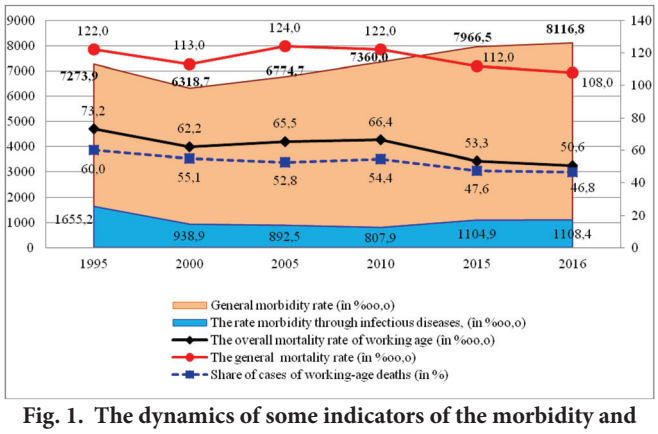


Fig. 1. The dynamics of some indicators of the morbidity and mortality rate, in the period 1995-2016.

[Source: made up by the author, on the basis of the data: http://www.statistica.md/category.php?l= ro&idc=198 and the database on communicable diseases CNSP: www.cnsp.md, years 1995-2016]

omitted (no less important), manifested through the incoherence of adjustment of the organizational forms and the insufficiency of human potential in achieving the increased number of tasks of the Service, stipulated in the new health policies [13,14,15]. Thus, the results of the study conducted in the Service, show a presence of a functional load per staff unit 1.2-1.4 bigger that the average value per country in over 62.8% of institutions, with an uneven territorial distribution (fig. 2). Functional overload is determined, first of all, by staff insufficiency, occurred by its exodus due to low and unattractive wages during the last 20 years. The impact of this phenomenon, first of all, negatively affects the indicators of quantitative and qualitative coverage of public health services provided to the population. Secondly, the efficiency of services provided under these conditions, do not correspond to the principles of economic and effective management of institutions, as well as to the requirements recommended by international bo-dies.

Thus, it has been established that in the territories where the number of hygienically supervised units is lower than the national average (1061 units) – the prime cost of a unit

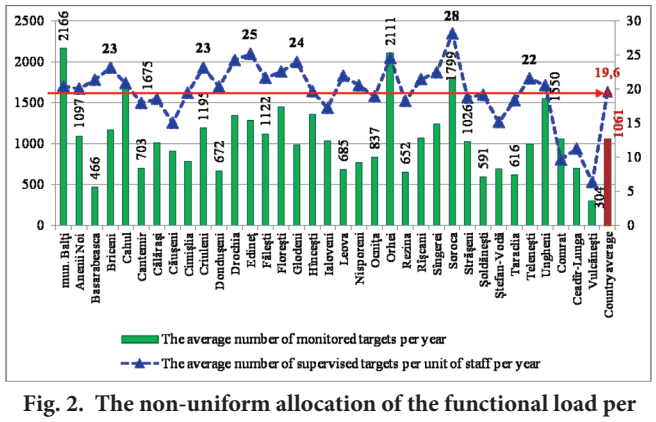


Fig. 2. The non-uniform allocation of the functional load per unit of personnel in the territorial PHC, (except PHC of mun. Chișinau, average and absolute data).

[Source: made up by the author based on statistical multiannual data (form18), NCPH years 2005-2016]

of state surveillance of public health (in lei/per unit of supervised objective) is much higher comparatively to the national average (2674.2 lei) (fig. 3). Territorial difference of prime cost of state surveillance activities in public health, accompanied by a functional load lower than the national average, along with unfavorable indices of health, denote the presence of management problems, determined by inefficient expenditures admitted in 31.4% of territorial institutions.

Improvement of these indicators predominantly requires the implementation of a new paradigm and new principles of functioning of the health system, oriented "de facto" towards the prevention and prophylaxis of diseases, the widespread implementation of measures that differ from the prophylaxis of communicable diseases.

It is well-known, that non-communicable diseases, through their etiology and manifestation, have a polyetiologic aspect of genesis, which is largely determined by the complex impact of environmental risk factors, by the determinants of health, as well as by individual behavior. In addition to this, for non-communicable pathologies it is characteristic a latent evolution and, most often, a late diagnosis.

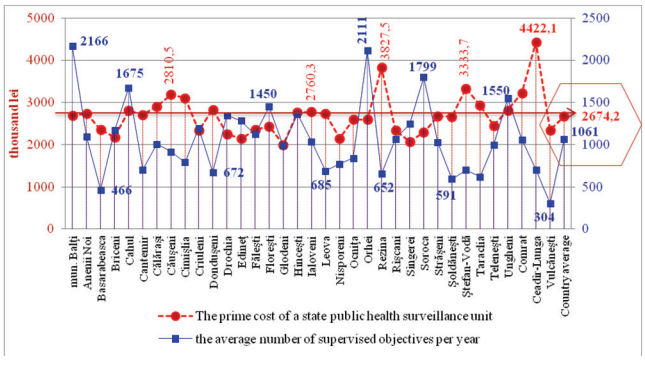


Fig. 3. The prime cost of a state public health surveillance unit in terms of administrative territories (except CPH Chisinau), (in abs. values, thousand lei and surveillance units).

[Source: made up by the author based on statistical multiannual data (form 18), NPHC years 2005-2016]

The epidemiology of non-communicable diseases, their prevention and prophylaxis, requires a rather complex approach and a coherent normative and organizational-methodological support. According to the Law No 10, art. 50, point 3 "...the primary actions of prevention and control of non-communicable diseases are aimed at supporting the aspirations of individuals and the community for assuring and forming a healthy living behavior" – we would add, "with early identification and prevention of the harmful impact of health risk factors". Actually, there is a need for individual awareness of responsibility for one's own health.

It is necessary to note, that nowadays the range of environmental risk factors for health, includes all areas of activity and behavior of the population. Thus, the call of WHO and the specialists in public health, for the application of the

principle "Health in All Policies", "comes to create a potential for improving the health of the population by exploiting the energy of multiple areas of interest through intersectoral governance" [16,17]. Realizing the idea of this principle or postulate, the state/society is going to create coherent conditions in the sphere of development of capacities and complex interventional structures for health protection, institutional capacities that would promote a healthy living and would educate the population about the need of being healthy. Furthermore, based on the fact that health is not only the prerogative and responsibility of the health system, the state/society has to create conditions for a broad perception of health as its "treasure", as a driving force for the sustainable development of the country [18]. Therefore, the individual's health is a "good" of his own, as well as of society – and it has a price. This "good" also requires investment – investing in the "healthy man". Investing in healthy people contributes substantially to reducing the economic burden of disease on society. In this context we would like to recall that, the establishment of mandatory health insurance (Compulsory Medical Insurance) provided allowances for those who were healthy (for prophylactic measures in volume of 4% out of Compulsory Medical Insurance Fund), which subsequently, through certain administrative mechanisms and "interventions", were diminished and / or partially used inefficiently.

According to respondents' opinions, financing medical institutions according to the principle "...depending on the number of patients treated and the number of visits to the family doctor", nowadays does not contribute to the motivation of specialists and corresponding structures to have fewer patients or a healthy society. Increased investment of 11.2 times of the public health budget, compared to 2004[16], did not contribute to a significant improvement of population health indices (fig. 4).

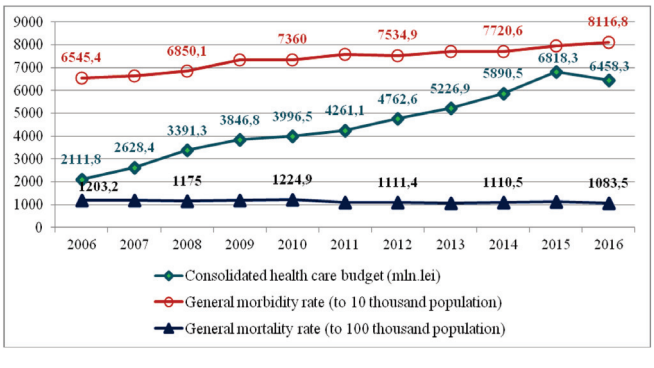


Fig. 4. Dynamics of morbidity and mortality rates in relation to the public health budget (years 2006-2016).

[Source: drawn up by the author based on data www.stratistica.md]

Overall morbidity in this period increased by 18.1% (compared to 2004), and the overall mortality rate is "a contour line" with a slight decrease, or reduction by one case to one hundred persons (respectively, from 1160.0 to 1083.5 at 100 thousand persons). Consequently, it is considered relevant to propose adjusting this principle of financing sanitary institutions, including by increasing the annual number of healthy people, or the growing annual share of the healthy population – who, due to preventive and prophylactic measures, did not need to address for medical assistance.

Also respondents consider, that state budget allocations for public health activities, or activities for the implementation of disease prevention and prophylaxis measures, are not objectively adjusted to the health status of the population. So, the ratio between the budget for mandatory health insurance for patients and the budget of public health surveillance institutions, aimed at implementing preventive/prophylactic measures for diseases and promoting health, the latter was on average 30.3 times smaller, constituting in 2016 only 2.7% (fig. 5). Here, we must point out that its value is 3-6 times smaller compared to the share of public health spending in some countries (Romania – 7%, Italy – 9%, France – 10%, Russian Federation – 10%, Germany – 14% and USA – up 18%).

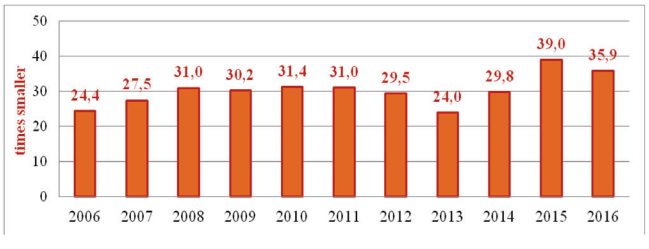


Fig. 5. The difference (times smaller) of the budget aimed at implementation of measures of prevention and prophylaxis of diseases – compared to the budget for medical care for patients.

[Source: drawn up by the author based on data www.stratistica.md]

Therefore, the results of the opinion poll of the Service managers, and also the resulting medical-social indices (of the researched period), point to the need to carry out institutional reforms to adjust the state health surveillance system and financial support for disease prevention and prophylaxis activities to the current conditions.

A regrouping of professional resources and functional efforts of the Service is required, oriented towards consistent adjustment of the structures for operation under newly-created conditions in society. In this context, respondents propose a restructuring of the effort (tab. 2), setting in the forefront the strengthening of health promotion activities among the population (response rate of 39.4±5.8%), surveillance and prophylaxis of communicable diseases (with a rate of 27.6±5.3%) and the organization of immunoprophylaxis (response rate of 19.7±4.7%), followed by the achievement of the target activities for prevention and prophylaxis of non-communicable diseases (the summary response constituting a rate of 18.3±4.6). The latter activities are required to be based on a profound analysis of health state versus risk factors, applying sampled study programs instead of collect-

[16] Compared with the initial period of implementation of compulsory health insurance (year 2004)

Table 2

Respondents' opinion on public health measures considered as priorities for planning and implementation in the territory

Rank according to respondents	Priority measures (in domains)	Rate of response to the 8 variants (%±m)	Share of cases of response (%)	Number of answers opted for 8 variants (abs.)
I	Promoting health	39.4±5.8	24.1	28
II	Surveillance and prophylaxis of communicable diseases	27.6±5.3	16.4	19
III	Immunoprophylaxis of decremented contingents	19.7±4.7	12.1	14
IV	Surveillance and control of non-communicable diseases	18.3±4.6	11.2	13
V	Quality of drinking water and sewerage	15.5±4.3	9.5	11
VI	Children and young people's health / rational nutrition	11.3±3.8	6.9	8
VII	Ensuring proper management of the institution	11.3±3.8	6.9	8
VIII	Other measures	21.1±4.8	12.9	15
	Total	-	100.0	116

[Source: made up by the author based on managers' opinion data]

ing and analyzing routine data separately.

The respondents also propose to strengthen and improve the institutional management of the Service (by 11.3±3.8%), including by ensuring a consistent funding of workload and tasks, also by equitable remuneration of the staff at the level of healthcare specialists. The massive exodus of specialists from the Service, as a result of insufficient and unattractive remuneration, plus inefficient personnel policy applied in the field, can ultimately compromise the achievement of the 10 operational public health tasks; endanger the epidemiological safety of the country, as well as its sustainable development.

Conclusions

The functioning of the State Service of Population Health Surveillance, under conditions of an austere budget and dispersion of limited resources to the existing number of institutions, does not contribute to efficiency in management of public health surveillance according to the standards and recommendations of international bodies.

Sanitary-hygienic and antiepidemic activities carried out in the researched period, have contributed to epidemiological stability of communicable diseases accompanied by a significant change in the structure of general morbidity, placing in the foreground morbidity and mortality through non-communicable diseases. Simultaneously, it was found that the activities carried out by the specialists of the Service on the prevention of non-communicable diseases, were and are mainly focused on control actions, restrictions (prohibitions) and constraints, which do not lead to diminishing their morbidity in society.

Research of the health state in relation to the factors that determine it, at the moment, is deviating from the classical methodology, the reason for which is the lack of institutional capacities and potential capable of carrying out sampled research and complying with the unanimously accepted regulations.

Analyzing the dynamics of statistics data, the health system's indicators related to the health status of the population, activities based on their financing according to the principle «... depending on the number of patients treated and the number of visits to the family doctor», the latter does not contribute to the motivation of specialists and structures to have fewer patients, or does not contribute to disease prevention and / or improving health of the population. Society faces an accumulation of chronically ill people on a background of diminishing primary registered cases of diseases.

The results of the study, as well as managers' opinion, point out the necessity of regrouping the professional resources and the functional effort of the State Service of Public Health Surveillance, with the adjustment of institutional structures for functioning according to identified priorities, the health state of the population and an equity in the allocation of budget resources to the Service, as well as for ensuring a coherent motivation of the staff and a fair protection of the health of the population.

References

1. Legea privind supravegherea de stat a sanatatii publice nr. 10-XVI din 03.02.2009 [Law no. 10-XVI of 2009 Feb 3, regarding public health surveillance]. Monitorul Oficial al Republicii Moldova. 2009 April 3;(67):art. no. 183. Romanian.
2. Centrul Natonal de Sanatate Publica. Jaloanele edificarii sanatatii publice in Republica Moldova: 65 ani [Highlights of the public health establishment in the Republic of Moldova: 65 years]. Bahnarel I, Nistor I, Filip A, Cravet P, editors. Chisinau; 2013. 256 p. ISBN: 978-9975-4027-1-2. Romanian.
3. Istoriia sanitarno-epidemiologicheskoi sluzhby [History of Sanitary Epidemiological Service] [Internet]. Moscow: ITAR-TASS News Agency; 2013 [cited 2017 Dec 18]. Available from: http://tass.ru/spravochnaya-informaciya/695181. Russian.
4. Legea Republicii Moldova nr. din 16.06.1993, privind asigurarea sanitaro-epidemiologica a populatiei [Law of the Republic of Moldova no. 1513 of 1993 June 16, on sanitary-epidemiological insurance of the population]. Monitorul Oficial al Republicii Moldova. 1993 Oct 30;(009):art no. 263. Romanian.
5. Legea nr.175 din 21 iulie 2017 pentru modificarea si completarea

unor acte legislative [Law no.175 of 2017 July 21 for the amendment and addition of some legislative acts]. Monitorul Oficial al Republicii Moldova. 2017 Aug 18;(301-315)(6224.6238): art. no. 335. Romanian.
6. Thomson S, Figueras J, Evetovits T, et al. Economic crisis, health systems and health in Europe – impact and implications for policy. Maidenhead: Open University Press; 2015. 218 p. eISBN: 978-0-335-26401-8. (European Observatory on Health Systems and Policies series).
7. MacLehose L, McKee M, editors. Health care systems in transition: Republic of Moldova. European Observatory on Health Care Systems (Copenhagen). 2002;4(5):19. [Cited 2017 Dec 18]. Available from: http://www.euro.who.int/__data/assets/pdf_file/0006/95163/e81265.pdf
8. Hotararea Parlamentului nr. 597 din 03.10.1995, privind aderarea Republicii Moldova la Organizatia Mondiala a Sănătății (OMS) [Decision of the Parliament no. 597 of 1995 Oct 3, on the accession of the Republic of Moldova to the World Health Organization (WHO)]. Monitorul Oficial al Republicii Moldova. 1995 Oct 19;(5)8:art. no. 644. Romanian.
9. Biroul National de Statistica al Republicii Moldova [National Bureau of Statistics of the Republic of Moldova] [Internet]. Chisinau: Biroul National de Statistica [cited 2018 Jan 6]. Avalable from: http://www.statistica.md/category.php?l= ro&idc=198; www.cnsp.md.
10. Georgieva L, Burazeri G, editors. Health determinants in the scope of new public health. Sofia: Hans Jacobs Publishing; 2005. 633 p. ISBN 3-89918-146-8.
11. Ministerul Sanatatii al Republicii Moldova, Centrul National de Sanatate Publica, et al. Studiu de indicatori multipli în cuiburi (MICS): Republica Moldova: Raport final [Multiple indicator cluster survey (MICS): Republic of Moldova: Final report]. Chisinau: Elan Poligraf; 2015. 324 p. ISBN 978-9975-66-441-7. Romanian.
12. Ministerul Sanatatii al Republicii Moldova; Serviciul de Supraveghere de stat a Sanatatii Publice; Centrul National de Sanatate Publica. Supravegherea de stat a sanatatii publice in Republica Moldova: Raport national, 2014 [State Public Health Surveillance in the Republic of Moldova: National report, 2014]. Bahnarel I, Pantea V, Salaru I, Pisla M, et al., editors. Chisinau: CNSP; 2014. 200 p. Romanian.
13. Hotărîrea Guvernului nr. 384 din 12.05.2010, cu privire la Serviciul de Supraveghere de Stat a Sănătății Publice [Government Decision no. 384 of 2010 May 12, on the State Public Health Supervision Service]. Monitorul Oficial al Republicii Moldova. 2010 May 25;(78-80):art. no. 455. Romanian.
14. Politica Nationala de Sanatate. Hotararea Guvernului nr. 886 din 06.08.2007 [National health policy. Government Decision no. 886 of 2007 Aug 6]. Monitorul Oficial al Republicii Moldova. 2007 Aug 18;(127-130):art. no. 931. Romanian.
15. Strategia nationala de sanatate publica pentru anii 2014-2020. Hotărarea Guvernului nr.1032 din 22.12.2013 [National public health strategy for 2014-2020. Government Decision no.1032 of 2013 Dec 22]. Monitorul Oficial al Republicii Moldova. 2013 Dec 27;(304-310):art. no. 1139. Romanian.
16. Lillvis DF, Greer SL. Strategies for policy success: achieving 'good' governance. In: Greer SL, Wismar M, Figueras J, editors. Strengthening health system governance: better policies, stronger performance. New York: Open University Press; 2016. p. 57-84. (European Observatory on Health Systems and Policies series).
17. Mossialos E, Permanand G, Baeten R, Hervey T, editors. Health systems governance in Europe. The role of European Union law and policy. New-York: Cambridge University Press; 2010. 762 p. ISBN 978-0-521-74756-1.
18. Suhrcke M, Rocco L, McKee M. Health: a vital investment for economic development in Eastern Europe and central Asia. Copenhagen: WHO; 2007. 249 p. ISBN 978-92-890-7282-3.

DOI: 10.5281/zenodo.1186196
UDC: 616.995.132-053.2

Evolution of the toxocariasis monoinvasion in comparison with the toxocariasis associated with other parasites in children

***Placinta Gheorghe**[1,2], MD, PhD, Associate Professor;
Stirbu Tatiana[1,3], MD, Assistant Professor; **Tovba Lidia**[4], MD, Assistant Professor

[1]Department of Infectious Diseases, [4]Department of Infectious, Tropical Diseases and Medical Parasitology
Nicolae Testemitsanu State University of Medicine and Pharmacy
[2]Consultation and Diagnostic Center of Medical Parasitology and Tropical Diseases
Toma Ciorba Republican Hospital of Infectious Diseases
[3]Clinical Municipal Hospital of Infectious Diseases in Children, Chisinau, Republic of Moldova
*Corresponding author: gheorghe.placinta@usmf.md. Received January 11, 2018; accepted February 26, 2018

Abstract

Background: Toxocariasis is a parasitic infection with a major risk to children, especially because of their incompletely developed immune system, high risk of infection or frequent re-infection, all correlated with living standards and personal hygiene. Toxocariasis occurs most frequently occult. However, evident clinical manifestations may be found, due to the migration of larvae in the second stage of development, the degree of toxocara invasion and the immune system of the child.

Material and methods: The study presents the evolution peculiarities in a group of 94 children with toxocara monoinvasion compared to a group of 73 children with the presence of two or more parasitoses. Clinical particularities, representative laboratory indices, treatment and its influence on clinical and paraclinical indices were examined.

Results: The presented article compared the most common clinical signs and paraclinic changes in both studied groups. Was examined the specific treatment for each group of patients and its action on the laboratory indices and especially the influence of treatment on the antibody titer to *T. canis*.

Conclusions: The most common clinical signs were asthenia, weight loss. An increase in eosinophil level is recorded only in the 13.5% in the cases of Toxocara monoinvasion and in 15.1% of the cases with Toxocariasis associated with other parasites. Anti-toxocara specific therapy proved to be much superior to other medications with a significant reduction in the percentage of eosinophils and total IgE.

Key words: toxocariasis in children, larva migrans visceralis.

Introduction

The term Larva Migrans Visceralis (LMV) was first used by Beaver et al. [1] in 1952, when the authors reported the clinical picture of three children with chronic marked eosinophilia, hepatomegaly, pulmonary damage, fever, and cough, all those listed being produced by larval migration in lungs with subsequent migration to other organs. Beaver et al. [1] used the term LMV to define larval migration in the second phase of development in the organs of the intermediate host (humans) [2].

T. canis and *T. cati* are found throughout the globe with a higher frequency in developing countries with a poor sanitation system. The human genetic tendency to be surrounded by pets (dogs and cats) also has a decisive factor in the spread of this infection [3].

Toxocara genus belongs to the class Nematoda, order Ascaridoidea, family and subfamily Ascarinae and includes 21 species. The species *T. canis, T. cati,* and *T .leonina* are most commonly implicated in LMV syndrome [4], in humans this syndrome is caused by *T. canis* and *T. cati* [3].

Mature worms live an average of four months, after six months almost all are eliminated spontaneously from the body [4]. The *T. canis* female produces almost 200,000 eggs [5], which are sufficiently resistant and can survive for a long time in soil [4]. The eggs are not embryonated in faeces so they are not infectious. Only temperatures of 15-30 ° C plus humidity are necessary conditions for the eggs to be embryonated and become infectious within 2-5 weeks after elimination [6].

The definitive host for *T. canis*, is the domestic dog, where the adult worm populates the animal's intestinal small intestine [3]. The elimination of faeces in public spaces by dogs contributes to the zoonotic spread of parasitosis [5].

Infection in children occurs through the ingestion of *T. canis* embrionated eggs [6] by direct contamination of the hands, especially from the contact with puppies aged between 2 weeks and 6 months and by indirect contact with contaminated objects inside or outside the house.

LMV is a syndrome caused by the ingestion of soil infected with *T. canis* eggs [7-9]. Various studies have attempted to make a statistical link between the high risk of developing toxocariasis in children and various poor childhood habits. Some authors have reported the presence of pica sdr. in children with toxocariasis with Larva Migrans Visceralis syndrome [11-17] most often in the age group of 1 to 6 years, with a slight prevalence of boys over girls [10]. Two contradictory studies have shown, on the one hand, a relationship between the habit of chewing nails and toxocariasis [18], while the other showed the absence of this relation [7].

The presence of a dog in the house has also been classified

as a risk factor for toxocariasis according to some studies [8,10,19,12,13,20,21]. However, some authors have pointed out that if hygiene measures are kept, this correlation is not maintained [8]. Several studies have provided information on the relationship between seropositivity at *T. canis* in puppy owners, who had their pets for at least 3 months [7,20]. Iddawela et al. have demonstrated that socio-economic status is not an increased risk for toxocariasis [20]. Other authors have shown the relation between this parasitosis and the socio-economic status with such indicators as low income and lack of education [5,18,22]. They have found a connection between the high prevalence of toxocariasis and the low level of urbanization or the lack of access to sanitary conditions [8,18,23]. Thus, the many studies performed in this field had contradictory results, but all of them have a common side – toxocariasis presents a very varied seroprevalence: from 9.7% to 43% in children in various areas of the world [20-22, 24-27]. In most cases of invasion with *T. canis*, infection occurs asymptomatically (approximately 44.4% of cases) [28], the systemic manifestations reaching only 15.5% of diagnosed cases [29].

Because of the variability of clinical signs, a new classification of toxocariasis was proposed in the 1992-1993, according to which the disease was divided into 3 clinical forms: LMV (Larva Migrans Visceralis), OT (Ocular Toxocariasis) and occult form of toxocariasis [30, 31].

The proposed classification was presented as a compromise between clinical observations of patients, the presence of immunopathogenic mechanisms including the degree of immunological response and the location of the toxocara larvae. In fact, this classification divides toxocarosis into the classical, systemic, occult form, and compartmentalized (ocular and neurological) forms. The last two forms are likely to be classified separately, being the last penetration sites of the Toxocara larvae [32].

LMV has been described as a syndrome with marked manifestations of hypereosinophilia, hepatosplenomegaly, fever, hypergammaglobulinemia [1], leukocytosis, manifestations that occur in children from 1 to 5 years, with an average duration of 2 years [33].

Various authors have found a correlation between the presence of anemia and toxocariasis [1,14, 25, 35, 36, 37, 38]. Others, (Glickman et al.) found a correlation between a leukocytosis of 10 x 10^9 and a positive ELISA for *T. canis* [34].

Ocular toxocariasis is a clinical form that affects with the same frequency women and men and occurs at an early age. In literature OT is described as having a frequency from 0 to 10% [21, 28, 39] with an average age at the time of occurrence from 3 to 11 years [11, 40]. The disease is unilateral in most cases with a minimal or moderate degree of inflammation [41, 42]. Clinical manifestations are presented through peripheral granuloma of the retina in 50% cases, macula in 25%, and in 25% cases occurs endophthalmitis. Granuloma can also appear in the optic nerve [43]. Magnaval et al. [44], and Sabrosa and Souza [15] in 2001. reported that eosinophilia is usually absent in occult toxocariasis.

Throughout the history of the study of toxocariasis, have been described various types of systemic damage. It all began in 1952 when Beaver et al. [1] have described several clinical cases of toxocarosis with skin damage. In the same year Beaver described 3 cases of hepatomegaly in children with toxocariasis, one child also presented splenomegaly. These children endure liver biopsy, with an extensive area of liver necrosis and inflammation. The authors also found eosinophilic leukocytes as well as giant and epithelial cells around the areas of necrosis [1].

Other authors, studying the same pathology, did not find a direct correlation between splenomegaly [7] and hepatomegaly [34] in patients with toxocariasis, but demonstrated the presence of isolated hepatomegaly in patients with this parasitosis [7,45]. Studying the incidence of splenomegaly and hepatomegaly in patients with toxocariasis, in various studies the authors presented a rate of hepatomegaly between 11 and 85% [14,17,28] and splenomegaly between 20 and 45%. Despite of such diverse rates of hepatomegaly, a slightly elevated level of the liver occurred in nearly 90% of children with toxocariasis according to a study in Brazil [46].

Taylor et al. described abdominal pain as one of the most common symptoms, especially in children with high antibody titres of *T. canis* [40]. Iddawela et al. have assumed that the major cause of abdominal pain is mesenteric lymphadenopathy as a response of the intermediate host to the migration of the toxocara larvae [20].

Also, in several studies conducted in children with toxocariasis, there were found hypoecogenic lymph nodes with a diameter of up to 8 mm [47]. Two children with pancreatic lymph node were also described. In literature there are reports of liver abscesses in toxocariasis [48-50]. Between 1996 and 2002 three cases of pleuritis with positive ELISA were described for *T. canis* [51-53].

Some authors have demonstrated the conection between bronchial asthma and toxocariasis [45], others have insisted that this was possible in patients with atopic and / or allergic antecedents [54]. The literature includes multiple descriptions of bronchospasm-associated with toxocariasis in children [14, 21, 55, 33]. Alderets et al. describe the association of wheezing with positive serology for *T. canis* [18]. Other authors reported that such respiratory signs as cough are common in children with positive serology for toxocariasis [20, 45, 56, 57].

Material and methods

The study included 167 children aged from 3 to 18 years who were divided into two research groups: the first included 94 children diagnosed with toxocariasis monoinvasion, and the second group included 73 children, with toxocariasis associated with other parasitoses (ascaridosis, oxyuriasis, giardiasis). Patients were examined clinically, showing the most common clinical and paraclinical signs, the general

blood count, biochemical test (ALT and AST), total IgE, antibody titer against *T. canis*.

Results and discussion

The study involved 167 children with chronic visceral toxocariasis, 94 of them with toxocara monoinvasion and 73 in combination with various other parasitoses. The duration of the toxocara invasion ranged from 1 to 9 years, the majority of 117 (70.1%) with a duration of 2-7 years.

The age of children with toxocara monoinvasion was presented by next values: age category 4-7 years constituted 22.3%, 8-12 years 38.3% and 13-18 years 39.4%. In these patients the bronchopulmonary form prevailed in 32 children (34.0%) followed by the neurological form in 30 children (31.9%), cutaneous form in 16 children (17.0%), digestive form in 10 children (10.5%) and other clinical variants in 6 children (7.3%).

The spreading of the process demonstrated the involvement of a single organ system in the pathological process in 33 cases (35.1%), two systems in 39 cases (41.5%) and three and more systems in 22 cases (23.4%).

Among the children with toxocariasis associated with other parasitoses (ascaridiasis – 25 cases, oxyuria – 18, lambliasis 16, ascaridiasis with oxyuria – 5, ascaridiasis with lambliasis – 9), the age category 3-7 years constituted 16,4%, 8-12 years – 37.0%, and 13-18 years – 46.6%. The most common clinical form was neurological with involvement of 25 children (34.2%), followed by bronchopulmonary clinical form in 19 children (26.02%), digestive form in 14 children (19.2%), cutaneous form in 8 children (11%), and other manifestations in 7 children (9.6%). Clinical manifestations involving only one system were recorded in 26 children (35.6%), two systems – 32 children (43.8%), three and more affected systems were determined in 15 children (20.5%).

The most frequent clinical manifestations in the group with toxocara monoinvasion were headache and long-standing cough, both in 33% of cases, followed with a decrease in percentage value (from 25.5% to 20.3%) by hepatomegaly, vertigo, abdominal pain, diffuse echographic changes in liver, skin pruritus, sleep disturbances. The other 6 clinical signs with a percentage decrease from 20% to 10% were: maculo-papular rash, splenomegaly, neuropsychiatric disorders (impulsivity, inability to concentrate, poor memory, chronic apathy, etc.), physical asthenia and weight loss (fig. 1).

In the group with toxocariasis associated with other parasitoses the most common symptom was headache with 42.5% followed by abdominal pain in 32.9% of cases, vertigo in 31.5%, sleep disturbance, physical asthenia and long-standing cough in 27.4%. Thus, within 6 more frequent symptoms, 4 were with the involvement of central nervous system. These signs of CNS involvement were functional, with a gradual decrease to complete disappearance at different time intervals. In toxocariasis associated with other parasitoses, asthenia, weight loss, anorexia, pain and weight in the right hipocondrium were significantly more common compared to toxocara monoinvasion. Long-term cough (32.4% versus 24.7%) was reported more frequently in toxocara monoinvasion, whereas in toxocariasis associated with other parasitoses were found more frequent headache

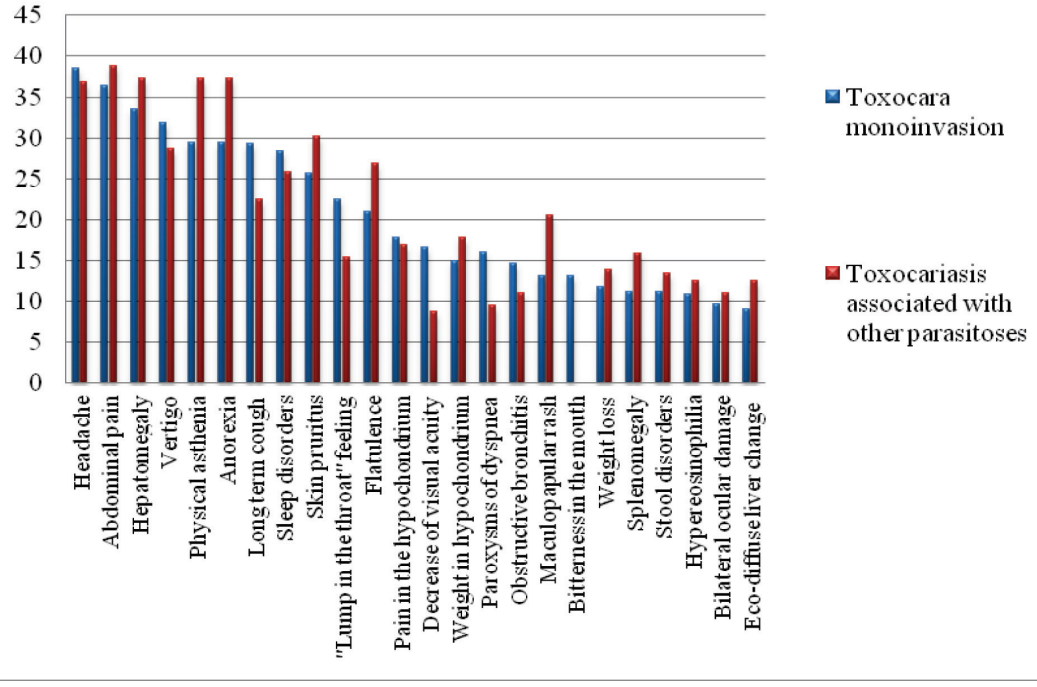


Fig. 1. Percentage distribution of the most common signs in children with chronic visceral toxocariasis in monoinvasion and in combination with other parasitoses.

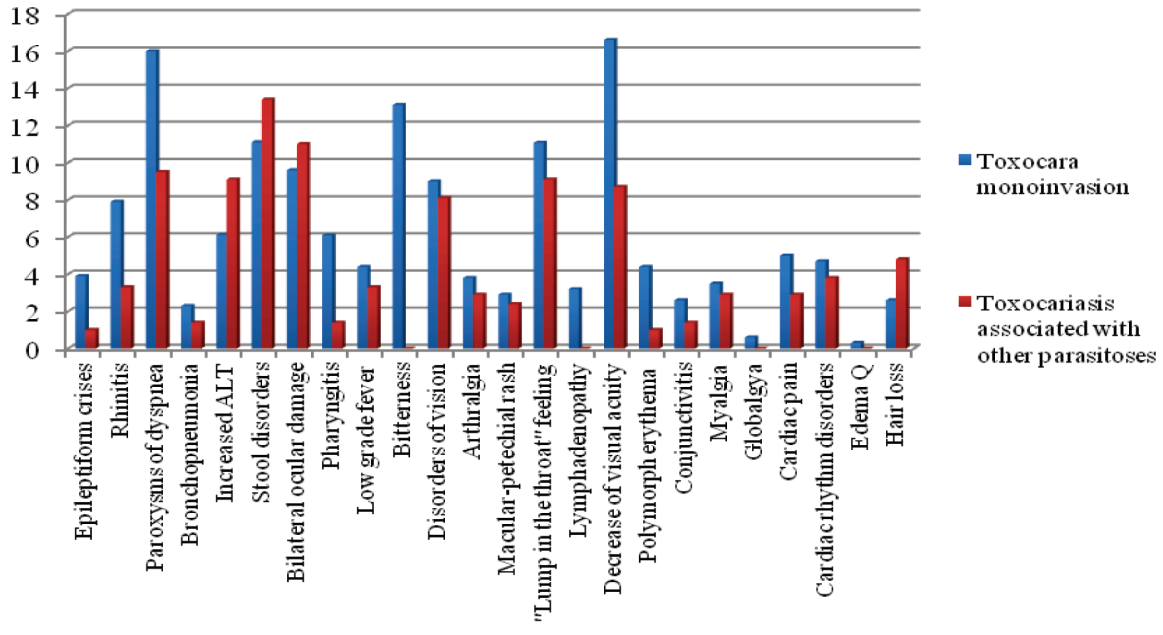


Fig. 2. Percentage distribution of rare signs in children with chronic visceral toxocariasis in monoinvasion and in combination with other parasitoses.

(42.5% vs. 32.4%), abdominal pain (32.9% vs. 21.6%) and vertigo (31.5% vs 24.3%) (fig. 2).

Clinical manifestations with an incidence of less than 10% during chronic toxocariasis in children are shown in figure 2. In 8.1% of cases of toxocara monoinvasions were recorded epileptiform seizures, signs of rhinitis and dyspnoea, in 6.8% of cases – bronchopneumonia, in 5.4% of cases elevated ALT level. In associated forms more frequently was observed an increased level of ALT (8.2%).

In the group of patients with associated diseases, nonspecific treatment included antiparasitic therapy (for ascaridiasis, lambliasis, enterobiosis) with a single dose of benzimidazole derivatives, the dose being repeated after 14 days only in oxyuria, a three days therapy being given in ascaridiasis and five days in lambliasis, in all cases being administered in only one administration per day. The specific antitoxocara treatment included in most cases benzimidazoles derivatives, being given twice per day with a dose of 10 mg / kg / body with a 10-14 days therapy.

The leukocyte count did not show significant deviations from the value of the norm in both groups, but during the surveillance it decreased significantly in the group of patients with specific treatment in the toxocara monoinvasion from 6.8 ± 0.47 to 5.5 ± 0.47.

Also, the number of erythrocytes increased compared to the values before treatment, being significant in the case of toxocara monoinvasion despite of the specific treatment applied. The percentage values of the lymphocytes had minor decrease compared to the baseline in the group with toxocara monoinvasion regardless to the applied therapy, but nevertheless remained above the mean values compared to the healthy individuals, and in the toxocariasis associated with other parasites this index increased from the initial values. The percentage of eosinophils, which was initially above normal values in healthy subjects (6.3 ± 0.61), decreased despite of the treatment applied: in the group with toxocara monoinvasion with anti-larval therapy up to 3.7 ± 1.0.

In the groups of children without specific treatment, both in the toxocara monoinvasion and toxocariasis associated with other parasitoses, the levels of ALT activity increased significantly compared to baseline values in healthy individuals. In mono-invasion they increased from 23.8 ± 2.4 to 62.8 ± 14.2 in the associated forms from 29.8 ± 4.24 to 45.5 ± 5.23. These liver enzyme behavioral findings demonstrate the direct involvement of toxocara larvae and their toxins in the development of hepatic cytolysis syndrome. At the same time, in both groups with specific treatment, ALT activity remained close to baseline mean values, not different from those seen in healthy individuals, demonstrating the safety of antilarvaric treatment in children.

Paradoxically, however, in both groups, regardless of treatment, antibody levels to *T. canis* increased, recording higher levels than those found up to treatment.

The total IgE level was much higher in the case of toxocara monoinvasion – 302.2 ± 41.0, compared to toxocariasis associated with other parasitoses – 187.6 ± 31.9. Specific treatment had a benefic effect on the evolution of total IgE, especially in the group of toxocariasis without comorbidities, recording significantly lower values compared to the baseline (145.4 ± 29.2 versus 302.2 ± 41.0). In the case of toxocariasis associated with other parasitoses, the total IgE level on a background of specific treatment decreased insignificantly, whereas in the group with toxocariasis without therapy, on the contrary, increased to 220.7 ± 43.2, compared to 187.6 ± 31.9.

The separate group analysis based on the specificity of the administered therapy revealed different behavior in the percentage distribution of clinical efficacy. A total of 56 (59.6%) children with toxocara monoinvasion were treated with anti-larvicidal drugs, while 38 children (40.4%) did not receive this treatment. In the course of chronic toxocariasis without comorbidities, a very pronounced clinical efficacy in children with anti-larvaric treatment was recorded in 16.1% of cases, whereas in the non-treated group only in 5.3% of cases. Clinical efficacy was also pronounced in a much higher proportion in the group of patients with anti-toxocara therapy – 48.2% of the 56 treated versus 23.7% of the 38 without treatment.

Of the 73 children with toxocariasis associated with other parasitoses, 52 received anti-toxocara therapy, while the other 21 followed only the anti-parasitic therapy of comorbidities. Very pronounced efficacy was found only in 7.7% of the 52 patients with treatment and in none of the children without anti-toxocara treatment. A clinical efficiency with a decrease in the proportion of 50-75% of the previous intensity of clinical signs was found in most patients with anti-toxocara treatment – 51.9% compared to 0.0% in those without the treatment. Also, partial clinical improvement was more frequent in the group of children with toxocariasis with anti-larval treatment with 19.2% of cases, compared with 4.8% of cases in children with therapy only against other parasitoses. The clinical ineffectiveness of anti-toxocara specific therapy was established in 21.2% of cases and in 95.2% in children who only followed treatment against parasitoses identified as comorbidities.

The level of antibodies to *T. canis* in children with toxocara monoinvasion who received specific treatment decreased to 31 (55.4%) compared to 18 (47.4%) found in those without specific therapy. In toxocariasis associated with comorbidities, the decrease in the level of the antibodies was 34 (65.4%) in the group with specific treatment and only in 2 children in the absence of this therapy. However, in both groups after anti-toxocara treatment it was noted the increase in antibody levels in approximately 1/3 of patients: 39.2% of cases from toxocara monoinvasion group and 30.8% from the group of toxocariasis associated with other parasitoses, although most of these children were with clinical improvement.

Conclusions

1. Clinical manifestations during chronic toxocariasis in children are very numerous (over 40 clinical signs), varying in intensity and incidence, the most common of them being headache, dry cough, abdominal pain.

2. In toxocariasis associated with other parasitoses, asthenia, weight loss, anorexia, pain and weight in the right hippocondrium were significantly more common compared to toxocara monoinvasion.

3. Blood hypereosinophilia is recorded in every 6th, 7th of the studied children, with a rate of 13.5% in the group with toxocara monoinvasion and 15.1% in the group with toxocariasis associated with other parasitoses.

4. Specific therapy in chronic visceral toxocariasis in children, both in cases of isolated toxocariasis and in combination with other parasitoses, had far superior clinical performance compared to other medications, including concomitant therapy for the eradication of parasites.

5. Specific treatment significantly reduced the percentage of eosinophils in both groups of patients, the IgE level in the toxocara monoinvasion group, whereas in the group of toxocariasis associated with other parasitoses in the absence of anti-larvicidal treatment both IgE total and the percentage of eosinophils increased.

References

1. Beaver PC, Snyder CH, Carrera GM, Dent JH, Lafferty JW. Chronic eosinophilia due to visceral larva migrans: report of three cases. Pediatrics. 1952;9:7-19.
2. Beaver PC. The nature of visceral larva migrans. J Parasitol. 1969;55:3-12.
3. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003;16:265-72.
4. Abe-Jacob CM, Oselka GW. Toxocaríase na infância [Toxocariasis in childhood]. Pediatria. 1991;13:48-55. Portuguese.
5. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. Epidemiol Rev. 1981;3:230-50. 6. Bourke GM, Yeates FM. Blindness due to household pets. Med J Aust. 1961;48:12-4.
7. Figueiredo SD, Taddei JA, Menezes JJ, Novo NF, Silva EO, Cristóvão HL, et al. Estudo clínico-epidemiológico da toxocaríase em população infantil [Clinical-epidemiological study of toxocariasis in children]. J Pediatr (Rio J). 2005;81:126-32. Portuguese.
8. Anaruma Filho F, Chieffi PP, Correa CR, Camargo ED, Silveira EP, Aranha JJ, et al. Human toxocariasis: a seroepidemiological survey in the municipality of Campinas (SP), Brazil. Rev Inst Med Trop São Paulo. 2002;44:303-7.
9. Mizgajska H. The role of some environmental factors in the contamination of soil with Toxocara spp. and other geohelminth eggs. Parasit Int. 1997;46:67-72.
10. Glickman LT, Chaudry IU, Costantino J, Clack FB, Cypess RH, Winslow L. Pica patterns, Toxocariasis, and elevated blood lead in children. Am J Trop Med Hyg. 1981;30:77-80.
11. Zinkham WH. Visceral larva migrans. A review and reassessment indicating two forms of clinical expression: visceral and ocular. Am J Dis Child. 1978;132:627-33.
12. Marmor M, Glickman L, Shofer F, Faich LA, Rosenberg C, Cornblatt B, et al. Toxocara canis infection of children: epidemiologic and neuropsychologic findings. Am J Public Health. 1987;77:554-9.
13. Ellis GS Jr, Pakalnis VA, Worley G, Green JA, Frothingham TE, Sturner RA, et al. Toxocara canis infestation. Clinical and epidemiological associations with seropositivity in kindergarten children. Ophthalmology. 1986;93:1032-7. 14. Snyder CH. Visceral larva migrans: ten years experience. Pediatrics. 1961;28:85-91.
15. Sabrosa NA, de Souza EC. Nematode infections of the eye: toxocariasis an diffuse unilateral subacute neuroretinitis. Curr Opin Ophthalmol. 2001;12:450-4.
16. Trabelsi S, Belhadj S, Kallel K, Zouiten F, Ben Becheur S, Ben Ayed N, et al. La toxocarose: une pathologie sous-estimée. A propos de 9 cas. Tunis Med. 2004;82:684-9.
17. Abe-Jacob CM. Contribuição para o estudo da toxocaríase na infância: aspectos clínico-laboratoriais de 40 casos [Contribution to the study of toxocariasis in childhood: clinical and laboratory aspects of 40 cases] [dissertation]. São Paulo: Universidade de São Paulo; 1990. Portuguese.
18. Alderete JM, Jacob CM, Pastorino AC, Rubinsky-Elefant G, Castro AP, Fomin AB, et al. Prevalence of Toxocara infection in schoolchildren

from the Butantã region, São Paulo, Brazil. Mem Inst Oswaldo Cruz. 2003;98:593-7.
19. Coelho LM, Silva MV, Dini CY, Giacon Neto AA, Novo NF, Silveira EP. Human toxocariasis: a seroepidemiological survey in schoolchildren of Sorocaba, Brazil. Mem Inst Oswaldo Cruz. 2004;99:533-7.
20. Iddawela DR, Kumarasiri PV, Wijesundera MS. A seroepidemiological study of toxocariasis and risk factors for infection in children in Sri Lanka. Southeast Asian J Trop Med Public Health. 2003;34:7-15.
21. Souza FA. Parâmetros clínicos laboratoriais na evolução de 104 crianças portadoras de larva migrans visceral por Toxocara canis [Clinical laboratory parameters in the evolution of 104 children with visceral larva migrans by Toxocara canis] [dissertation]. São Paulo: UFESP; 1992. Portuguese.
22. Kanafani ZA, Skoury A, Araj GF, El-Khoury M, Sawaya RA, Atweh SF, et al. Seroprevalence of toxocariasis in Lebanon: a pilot study. Parasitology. 2006;132:635-9.
23. Caseiro MM. Síndrome de Larva Migrans Visceral causada por Larvas de Toxocara canis, no município de Santos [Visceral larva syndrome caused by larvae of toxocara canis, in the municipality of Santos] [dissertation]. São Paulo: Universidade de São Paulo, Faculdade de Medicina; 1996. Portuguese.
24. García-Pedrique ME, Días-Suárez O, Esteves J, Cheng-Ng R, Araujo-Fernández M, Castellano J, et al. Prevalencia de infección por Toxocara em pré-escolares de una comunidad educativa de El Moján, Estado Zulia, Venezuela. Resultados preliminaries [Prevalence of Toxocara infection in preschool children from an educational community in El Moján, Zulia State, Venezuela. Preliminary results]. Invest Clin. 2004;45:347-54. Portuguese.
25. Malla N, Aggarwal AK, Mahajan RC. A serological study of human toxocariasis in north India. Natl Med J India. 2002;15:145-7.
26. Huerga Aramburu H, López-Vélez R. Estudio comparativo de la patologia infecciosa em niños inmigrantes de distintas procedências [Comparative study of the infectious pathology in immigrant children of different origins]. An Pediatr (Barc). 2004;60:16-21. Portuguese.
27. Taranto NJ, Passamonte L, Marinconz R, de Marzi MC, Cajal SP, Malchiodi EL. Parasitosis zoonoticas transmitidas por perros en el Chaco salteño [Zoonotic parasitosis transmitted by dogs in Chaco Salteño]. Medicina (B Aires). 2000;60:217-20. Spanish.
28. Altcheh J, Nallar M, Conca M, Biancardi M, Freilij H. Toxocariasis: clinical and laboratory features in 54 patients. An Pediatr (Barc). 2003;58:425-31.
29. Loez Mde L, Martin G, Chamorro Mdel C, Mario Alonso J. Toxocariasis en ninõs de una region subtropical. Medicina [Toxocariasis in children of a subtropical region]. Medicine (B Aires). 2005;65:226-230. Spanish.
30. Nathwani D, Laing RB, Currie PF. Covert toxocariasis - a cause of recurrent abdominal pain in childhood. Br J Clin Pract. 1992;46:271.
31. Rasmussen LN, Dirdal M, Birkebaek NH. „Covert toxocariasis" in a child treated with low-dose diethylcarbamazine. Acta Paediatr. 1993;82:116-8.
32. Pawlowski Z. Toxocariasis in humans: clinical expression and treatment dilemma. J Helminthol. 2001;75:299-305.
33. Schantz PM. Toxocara larva migrans now. Am J Trop Med Hyg. 1989;41:21-34.
34. Glickman LT, Schantz PM, Cypess RH. Epidemiological characteristics and clinical findings in patients with serologically proven toxocariasis. Trans R Soc Trop Med Hyg. 1979;73:254-8.
35. Gónzalez MT, Ibañez O, Balcarce N, Nanfito G, KoZubsky L, Radman N, et al. Toxocariasis with liver involvement. Acta Gastroenterol Latinoam. 2000;30:187-90.
36. Amir J, Harel L, Eidlitz-Markus T, Varzano I. Lymphedema as a presenting sign of Toxocariasis. Infection. 1995;23:389-90.
37. Ishibashi H, Shimamura R, Hirata Y, Kudo J, Onizuka H. Hepatic granuloma in toxocaral infection: role of ultrasonography in hypereosinophilia. J Clin Ultrasound. 1992;20:204-10.
38. Almeida MT, Ribeiro RC, Kauffman WM, Maluf Júnior PT, Brito JL, Cristofani LM, et al. Toxocariasis simulating hepatic recurrence in a patient with Wilms' tumor. Med Pediatr Oncol. 1994;22:211-5.
39. Good B, Holland CV, Taylor MR, Larragy J, Moriarty P, O'Regan M. Ocular toxocariasis in schoolchildren. Clin Infect Dis. 2004;39:173-8.
40. Taylor MR, Keane CT, O Connor P, Mulvihill E, Holland C. The expanded spectrum of toxocaral disease. Lancet. 1988;1:692-5.
41. Oréfice F, Boratto LM, Silva HF. Presumível toxocaríase ocular: revisão de 30 casos (1978-1989); relato de dois casos atípicos [Ocular toxocariasis: review of 30 cases (1978-1989); report of two atypical cases]. Rev Bras Oftalmol. 1991;50:31-7. Portuguese.
42. Cochereau I. Infections ocularires [Eye infections]. Encycl Méd Chir - Maladies infectieuses (Elsevier, Paris) 2000;8-003-L-10. French.
43. Stewart JM, Cubillan LD, Cunningham ET Jr. Prevalence, clinical feature, and causes of vison loss among patients with ocular toxocariasis. Retina. 2005;25:1005-13.
44. Magnaval JF, Glickman LT, Dorchies P, Morassin B. Highlights of human toxocariasis. Korean J Parasitol. 2001;39:1-11.
45. Taylor MR, Keane CT, O'Connor P, Girdwood RW, Smith H. Clinical features of covert toxocariasis. Scand J Infect Dis. 1987;19:693-6.
46. Hassan MM, Farghaly AM, Gaber NS, Nageeb HF, Hegab MH, Galal N. Parasitic causes of hepatomegaly in children. J Egypt Soc Parasitol. 1996;26:177-89.
47. Baldisserotto M, Conchin CF, Soares Mda G, Araujo MA, Kramer B. Ultrasound findings in children with toxocariasis: report on 18 cases. Pediatr Radiol. 1999;29:316-9.
48. Pereira FE, Musso C, Castelo JS. Pathology of pyogenic liver abscess in children. Pediatr Dev Pathol. 1999;2:537-43.
49. Moreira-Silva SF, Pereira FE. Intestinal nematodes, Toxocara infection, and pyogenic liver abscess in children: a possible association. J Trop Pediatr. 2000;46:167-72.
50. Rayes AA, Teixeira D, Serufo JC, Nobre V, Antunes CM, Lambertucci JR. Human toxocariasis and pyogenic liver abscess: a possible association. Am J Gastroenterol. 2001;96:563-6.
51. Jeanfaivre T, Cimon B, Tolstuchow N, de Gentile L, Chabasse D, Tuchais E. Pleural effusion and toxocariasis. Thorax. 1996;51:106-7.
52. Sakai K, Hirasawa Y, Hashimoto A. A case of toxocariasis with eosinophil-rich pleural effusion. Nihon Kokyuki Gakkai Zasshi. 2002;40:494-8.
53. Ashwath ML, Robinson DR, Katner HP. A presumptive case of toxocariasis associated with eosinophilic pleural effusion: case report and literature review. Am J Trop Med Hyg. 2004;71:764.
54. Buijs J, Egbers MW, Nijkamp FP. Toxocara canis induced airway hyperactivity in mice. Agent Actions Suppl. 1990;31:75-80.
55. Paolillo F, Migliori C, Fornari M, Belloni C. Toxocariasi: descrizione di un caso [Toxocariasis: a case report]. Pediatr Med Chir. 1997;19:141-2. Italian.
56. Buijs J, Borsboom G, van Gemund JJ, Hazebroek A, van Dongen PA, van Knapen F, et al. Toxocara seroprevalence in 5-year-old elementary schoolchildren: relation with allergic asthma. Am J Epidemiol. 1994;140:839-47.
57. Radman NE, Archelli SM, Fonrouge RD, del V Guardis M, Linzitto OR. Human toxocarosis. Its seroprevalence in the city of La Plata. Mem Inst Oswaldo Cruz. 2000;95(3):281-5.

REVIEW ARTICLES

DOI: 10.5281/zenodo.1186176
UDC: 616.311.4-002.36

Phlegmon of the oral floor. Contradictions in diagnosis and treatment

***Levco Simion,** MD, Assistant Professor; **Scerbatiuc Dumitru,** MD, PhD, Professor

Arsenie Gutsan Department of Oro-Maxillo-Facial Surgery and Oral Implantology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

*Corresponding author: simion.levco@usmf.md. Received December 28, 2017; accepted February 12, 2018

Abstract

Background: Although the symptoms of oral phlegmon have been described before Hippocrates and Galen, there have been discrepancies in the diagnosis and treatment plan appreciation in patients with phlegmon of the mouth floor until now. Ludwig's angina accounts for less than 1% of all pathologies of maxillofacial surgery. In the pre-antibiotic era, 50% of patients died. At the moment, the mortality rate is below 10%. If the pathology is not treated, patients die in 100% of cases.

Data sources: This study was conducted on specialty literature analysis. We analyzed 45 books and 8 articles. The aim of the study is to compare different sources in which the phlegmon of the oral floor is described.

Discussion: The phlegmon of the mouth floor can involve only the unilateral spaces of the mouth floor, and the diffuse phlegmon of the mouth floor, also called Ludwig's angina, compulsory involves bilateral spaces of the mouth floor. Two bilateral incisions in the submandibular regions and one in the submental region is the most practiced surgical treatment. The infection is poly microbial, with a mixed flora: aerobic alpha and beta hemolytic streptococci, staphylococci and gram-negative bacilli, anaerobic bacteroides and peptostreptococcus. Usually, the flora is from the oral cavity and pharynx.

Conclusions: Patient intubation is the method of choice when it is possible. Aggressive antibiotic treatment needs to be taken as early as possible. Surgical treatment is required to be performed as early as possible. The number of incisions and their location are chosen depending on the situation.

Key-words: oral floor phlegmon, analysis, contradictions.

Introduction

The phlegmon of the oral floor is a potentially fatal pathological process that can lead to death in a few hours. It is one of the most serious affections of the maxillofacial area. It is a progressive cellulitis of the mouth floor, which starts from the submandibular space. Dental affections are the most common causes of pathology [11,26].

Although it was written about the symptomatology of the phlegmon of the mouth floor before Hippocrates and Galen, its best description was made in 1836 by Wilhelm Friedrich von Ludwig [1.17.7]. Ludwig was a German physicist. He described this pathology as bilateral [3]. He described a fast spread of inflammation with obstruction of the airway, resulting in a mortality rate of 60% [12]. The term "Ludwig's angina" was given by Camerer in 1837. The word angina comes from the Latin "angere", which means suffocation and Ludwig in the name of the one who described it for the first time. The fact that Ludwig died of neck inflammation in 1865 is an interesting fact. Other attempts to name this pathology were: "marbusstrangulatorius", "angina maligna", "Garrotillo" – Spanish version [1,7,10,17,21,28].

Ludwig abstained from the "scientifically founded" suggestion on the etiology of the disease, but he differentiated this pathology from other inflammation and its symptoms from idiopathic edema, different from salivary gland pathology [10]. Until 1796, the extraction of teeth that caused abscesses had been contraindicated; because they believed that the inflammatory process would spread, causing a severe pathology [10].

At first, it was considered as a complication of local anesthesia, administered to perform dental extractions. In 1943, Tschiassny elucidated how the inflammatory process spreads from the molars to the oral floor. Apexes of the lower molar roots are located under the mylohyoid muscle insertion, developing the infection in submandibular space [1].

In the pre-antibiotic era, 50% of patients died, and nowadays the mortality rate is below 10% [17,28]. It was thought that most deaths were caused by sepsis, but mechanical airway obstruction was the factor that led to asphyxiation. In 1942 Taffel and Harvey achieved success in reducing mortality to 2% when they anticipated the diagnosis and insisted on aggressive surgical treatment by performing large incisions of sublingual and submandibular spaces. These large incisions allowed positioning of the base of the tongue in an anterior-inferior position and ensured asphyxiation prophylaxis [1].

The phlegmon of the oral floor represents less than 1% of all pathologies of the maxillofacial surgery. If the pathology is not treated, the patients die in 100% of cases. [10] Even with a well-established treatment plan, the risk of death is 5% [32]. Dental extractions were reported as a causal factor

in 90% of cases. In 70-80 percent of cases, teeth 7 and 8 are incriminated [3,17,28,30]. It occurs most often in men aged 20-60 years [24]. More than 50% have polymicrobial development [30]. If not diagnosed and treated in time, the patient's death rate may reach 75% in the first 12-24 hours [32].

Other causal factors include: Compound mandibular fractures, soft tissue laceration, sialadenitis, tumor superinfestation, pharyngeal infections and tonsillitis, lingual piercing, osteomyelitis, otitis media caused by gun fire, paratonsillar abscess [4], iatrogenic [3], furunculus, infected thyroglossal cyst, sepsis [11], mastoiditis, traumatic penetration of oropharynx, lymphocele [15,28]. Although most infections occur in healthy individuals, there are predisposing factors such as diabetes, neutropenia, aplastic anemia, glomerulonephritis and immunodiagnosis [12,14,17].

Inflammation also spreads in the sublingual space, diffusing behind the mylohyoid muscles, between the hyoglossus and genioglossus muscles. Through this way, the infection spreads to the epiglottis and produces edema in this region. The mandible body and the hyoid bone limit the spread of edema. This limitation leads to increased upper, lower, and posterior edema. Infection can be spread through deep cervical fascia and produce cellulitis, that extends from the clavicle to the face [1,7,25,28]. Due to the location of the roots of these teeth below the insertion of the mylohyoid muscle, the periapical infections diffuse easily to the submandibular space, consequently progressing easily towards the sublingual and submental spaces [3,11,17,19]. True Ludwig angina involves both submandibular spaces and is life threatening, but also a few spaces of the mouth floor can be involved [30]. The infection in the submandibular region can spread lower, through deep cervical fascia to the mediastinum. Less common, the infection can spread to the carotid, pterigopalatine fossa, cavernous sinus [7].

Bacterial activity in this pathology leads to significant muscular necrosis. Suppuration is not observed, but it occurs in the late stages of the disease. This phenomenon occurs very quickly and does not respect any anatomical barrier [3].

The infection spreads in adjacent spaces even directly or through the lymphatic way. Anaerobic organisms are also isolated when there are gangrenous changes in the tissue, due to the mixed effect with interstitial pressure effect, hypoxia and the effect of bacterial exotoxins. A vital tissue serves as a place for the development of anaerobic organisms.

The tissue is diffuse infiltrated with neutrophils and dead histiocytes. Leukocytosis is usually with nuclear polymorphic leukocytes [2,5,22,25].

The predisposing factors are: low social status, poor oral hygiene, drug abuse, alcohol, diabetes, hiv/aids, oral transplants, aplastic anemia, immunosuppression, steroid therapy [4,5,31].

Other rare occurrences are: pleoplasms, chronic or acute tonsillitis, salivary calculi, lower alveolar nerve blockade [24,31].

The present symptoms of oral floor phlegmon can develop in a fast way and depend on the level of infection. Patients usually suffer from dental pain, fatigue, ear pain, confusion, fever, malaise, severe pain, hypersalivation and edema of the tongue, neck and submandibular region. These symptoms can progress and include dysphagia, odynophagia, salivary gland secretion disorders, dysphonia, trismus and difficulty in breathing. These patients have an open mouth and protrusion of the mandible, lifted up tongue and tough swallowing. Breathing can be extremely difficult. Subcutaneous emphysema may occur in soft tissues. Classically, the collection of pus is not clinically appreciated [1,20,21,22,24,27,28].

Bilateral development is always present in the diffuse phlegmon of the oral floor. Laryngeal or supraglottic edema may lead to voice changes. Collections of pus may be absent [23].

Patients can suffer from unpleasant taste of the mouth, crepitations in temporomandibular space or unilateral pharyngitis. From the history of the disease, questioned patients will frequently complain a few days after the dental extraction. During the extraction of the causal tooth, the patient feels a fetid smell. There is a trismus due to induration of submandibular space [11,25,31].

Before the era of antibiotics, chest pains were due to the spread of mediastinal infection. However, the phlegmon of the mouth floor is rarely encountered; its incidence has decreased in past years due to the development of antibiotics and dental prophylaxis. This happens most often in young adults with dental infections, but it can develop at any age. In children are diagnosed approximately ¾ of the cases. Although the pathology is considered to be non-purulent, 81% of the cases were described as purulent [6,16,25].

Patients with phlegmon of the oral floor are dehydrated, mostly because of two reasons:

1. Pain during dysphagia. 2. Because of increased toxicity, urination and sweating are increased and lead to fluid loss of the body. Patients should be encouraged to consume water and should be hydrated intravenously [2,20,31].

There is a bilateral edema placed above the suprahyoid muscle, which has a rough, non-fluctuating consistency and is painful when palpation is done. The mouth is half-open and the tongue in contact with the hard palate, with a visible edema of the oral floor. The tongue is placed superior and posterior, which can lead to asphyxiation. If the edema spreads to the pterygoid region it is difficult to open the mouth. The most prominent clinical signs present in this pathology are: difficulty of swallowing and breathing, chills and fever, increased salivation, restricted language movements and the impossibility of opening the mouth [3,14,16,20,26]. The skin is tense, glossy, hyperemic, consistent and is usually described as "woody" or called hardening of the muscle. The edematous area of the neck is firm, painful, non-fluctuating and does not show pastiness. The condition is always bilateral and patients are often unable to open their mouth and speak [4,16,31].

When inflammation is not treated, it may spread and causes a massive edema above the hyoid bone, in the neck, with the submandibular gland involved, a symptom called "bull neck" [4, 16]. The edema in the submental space produces the sensation of double chin. The skin is tense due to the accumulation of inflammatory exudates in the interstitial compartment, which makes the tissue pasty. Sensitivity to palpation is present [5].

The first sign of laryngeal edema is dyspnea that gets worse when the patient is lying down. The symptoms of dyspnea should be taken seriously because they represent a complete airway obstruction. As the edema progresses, dyspnea gets worse and asphyxia may occur. Dyspnea usually occurs in paroxysm and in 10-12 days of the disease, death occurs [5,10,14,21].

There is the symptom of the hot potato voice. The voice is inconspicuous, hyponasal, due to inflammation of the posterior wall of the pharynx [6,12,7]. It can also detect snoring during breathing [7].

Purulent collections whose walls were made of partially decomposed gangrenous mass in the muscles were found among the cases where the autopsy was allowed. The periosteum of the internal mandibular surface was detached from the bone [10].

The edema in the mouth floor can be so broad, that the tongue can block the mouth and the oropharynx and if left untreated, the infection can spread to the chest cavity. It may result in an abscess of the pericardium and the lungs or throughout the body as a septic shock. Patients may experience an extreme lethargy, dehydration, and shortness of the breath, and require immediate medical attention [27].

Since the diagnosis of oral phlegmon is clinically established, the primary role of imaging in the assessment is to observe the presence and level of narrowing of the airways. It includes also locating some abscesses that need to drain, identifying the presence of gaseous bubbles, assessing the spread of pathology in other areas such as retropharyngeal space and mediastinum, and looking for the source of the underlying odontogenic infection. Computed tomography should be extended to mediastinum to factor out secondary mediastinitis [6,13,21].

Computed tomography is the most common used imaging method in assessing patients with phlegmon of the mouth floor. Because of the fact that patients may have respiratory deficiencies in lying position, computed tomography should be done with caution [10,21]. Computed tomography illustrates most often the increase of edema in soft tissues or less, the accumulation of pus in sublingual and submandibular spaces. Orthopantomography presents which are the teeth of the apical abscess [25]. Contrast computed tomography has become a choice in patient assessment. It can detect deep cervical infections [26].

The lateral radiography of the neck shows the shadow of soft tissue edema and if the laryngeal edema is present then the epiglottis may look like a high raised toe – the epiglottis sign [6,17]. If the patient is not able to perform computerized tomography, ultrasound is used. It can show successfully where there is serous inflammation and where purulent collection is. It can be used also as a guide for draining the purulent collection [6,24].

In case of phlegmon of the mouth floor, the radiological report should include:

a) the presence, location and the spread of inflammatory processes; b) the presence and location of any fluid collection for drainage; c) the presence of gaseous bubbles in soft tissues; d) the presence and level of narrowing of the airways; e) if the collection has spread to mediastinum; f) detecting the source of the infection, if it is odontogenic [6].

Grodinsky proposed four criteria for making the difference between Ludwig's angina and other neck abscesses: 1) it is bilateral, 2) it produces gangrenous serum-blood infiltrate with or without pus, 3)it involves tissues, fascias, muscles but not glandular tissues, 4) it spreads by continuity, not by the lymphatic pathway [31].

The differential diagnosis is done with infiltrative carcinoma or sarcoma. In this case, the fever is not present and the odontogenic source is not determined [2]. The dermoid and epidermoid cyst, the thyroglossal duct cyst can mimic the abscess – especially when inflamed [6]. The abscess of the parotid gland, peritonsillitis, deep cervical suppurative nodules, angioedema and submandibular hematomas, can provoke diphtheria [11]. Differential diagnosis also confronts the tumors of the mouth floor that progresses slowly or with submandibular abscess, which is unilateral [17,22].

The edema of epiglottis can be caused by septicemia, upper airway obstruction. It can spread to parapharyngeal space and may pass into mediastinum causing bronchial erosions, mediastinitis, purulent pericarditis, tamponade, pneumothorax, pleurisy and empyema. Other possible complications are meningitis and vascular erosions. Some authors believe that these complications arise from a delayed diagnosis [1,2,3,21,22,24,7]. Other complications include cavernous sinus thrombosis, meningitis, cerebral abscess and suppurative encephalitis [4,10,21,28,29].

The spontaneous abscess bursting, or its rupture during manipulation can produce aspiration of pus, which leads to asphyxia, pneumonia or lung abscess. Spreading infection to the carotid artery may cause internal jugular vein thrombosis, carotid artery breakage or vocal cord paralysis. If the infection spreads to the spine, it can result in osteomyelitis and erosion of the spine, causing vertebral subluxation and spinal cord injury. The infection itself can pass into necrotizing fasciitis, sepsis and death. Horner's syndrome may occur and 9 out of 12 paralysis of the skull nerves due to the involvement of lateropharyngeal space [7].

Other complications: maxillary sinusitis, digestive tract disorder, carotid artery erosion, Grisel syndrome [3,11,15,16]. The complications of phlegmon of the mouth floor can be disastrous, the infection can spread to mediastinum, pericardial, peripleural, retropharyngeal, parapharyngeal spaces, and along large vessels [17,24,25].

Data sources

This research was done on the basis of literature analysis. We analyzed 45 books and 8 articles. The aim of the study is to compare different definitions and treatment plans in patients with phlegmon of the mouth floor. The article does not mention that the sources for the definition and the treatment plan are the same. The sources were found on the websites: www.books.google.com www.pubmed.com www.sciencedirect.com

Discussion

Contradictions in this pathology start right from the definition. Some authors consider that the phlegmon of the mouth floor can be both unilateral and bilateral, while others believe it is binding bilateral. We also find the term Ludwig's angina in the specialized literature. After analyzing specialty literature, we have selected the following definitions:

Diffuse phlegmon of the mouth floor – is a gangrenous, hypertoxic infectious process that includes submandibular, sublingual and submental spaces, with the tendency to spread in neighboring spaces. Some forms of the phlegmon of the mouth floor that evolve only in one direction [47, 48,52,53].

The phlegmon of the mouth floor is characterized by the involvement of two or more spaces located above or below the mylohyoid muscle [39,43].

Ludwig's angina – severe acute inflammation that spreads rapidly, bilaterally affecting sublingual, submandibular, submental spaces. Foreign literature [2,34,36].

Pseudo-Ludwig's angina – is the term used in cases of the phlegmon of the mouth floor of the non-odontogenic origin.

From these definitions, we can understand that Ludwig's angina corresponds to the diffuse phlegmon of the oral floor.

The infection is polymicrobial, with a mixed flora: aerobic alpha and beta hemolytic streptococci, staphylococci and gram-negative bacilli, anaerobic bacteroides and peptostreptococcus. However, the most common microorganisms are alpha and beta hemolytic streptococci. Usually, the flora is from the oral cavity and pharynx [1,4,3,17,19,21,24,7].

Anaerobic bacteria are permanently present and they may play a primary or synergetic role [3,10]. Anaerobic organisms are also isolated when there are gangrenous changes in the tissue, due to the combined effect with interstitial pressure, hypoxia, and the effect of the exotoxins produced by bacteria. A vital tissue serves as a place for the development of anaerobic organisms. E. coli - is also found in culture [5,10]. Candida and aspergillus species were also reported in a small number of patients [12].

Other bacteria detected in the inflammatory outbreak in patients with oral phlegmon are Bacteroidesmelaninogenicus, Bacteroidesoralis, Escherichia coli, Fusobacteriumnucleatum, Hemophilis influenza, Peptostreptococcus species, Spirochete species, Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus viridians, Clebsiella 15,16].

The distressing signs of streptococci infection are that infection tends to spread rapidly, leading to the release of exotoxins that destroy the intercellular substance and makes the spread of infection across tissues easier [5].

The most urgent treatment needs to be done to maintain vital functions [1,3]. The treatment is oriented in three directions: 1) maintaining breathing; 2) aggressive antibiotic therapy; 3) decompression of submental, sublingual and submandibular spaces [6].

The treatment is based on the combination of the following factors: early diagnosis, airway management, intense antibiotic treatment, extraction of the causal tooth, surgical treatment, incision and drainage of the involved areas, hospitalization and long-term surveillance [2,7,10]. The basic condition is to protect the airways. If patients are at risk of asphyxia, artificial respiration is ensured [1,4,8,9,21,28].

To perform the general anesthesia, the patient is intubated with fiber-optic laryngoscope when the patient is conscious and awake. In case of blind orotracheal intubation, nasotracheal intubation or tracheostomy is recommended, which should be an alternative method, in case orotracheal intubation is not possible [3]. Intubation is almost impossible in patients who, due to severe laryngeal edema, exhibit language tongue and trismus [1,4,5,10,14,15,23]. Tracheotomy should be avoided because tissue incisions at this level may lead to the spread of mediastinal hyperplasia. Tracheotomy is indicated when fiber optic insertion is impossible [11,29]. Often early tracheostomy may be the safest option for preventing airway obstruction. If the patient has signs of laryngeal edema like dyspnea, tracheostomy should be performed immediately to save patient's life. [1,4,5,10,14,15,23].

It is necessary to perform tracheostomy as low as possible to be as far away from the inflammatory process as possible [18,24].

The drawbacks of tracheostomy are: the landmarkers are hardly observable due to massive edema; anatomical formations are distorted due to edema. The infection can be spread due to the additional incision, the inflammatory process being adjacent to the tracheostomy, can penetrate the plague and cause pneumonia and tracheal stenosis. It was observed in 25 to 50 percent of cases in patients who had tracheostomy [4].

The use of the endotracheal tube has the following principles it allows: avoiding sedatives and narcotic drugs, but it disrupts breathing. The level of obstruction of the respiratory system can be better evaluated using the pulse oximeter. These parameters are observed depending on the clinical picture and vary from patient to patient [1,4,10,16].

Nasal intubation can lead to bleeding and abscess rupture [24,28]. In some situations it is recommended to use local infiltrative anesthesia, lidocaine of 2 percent, which is enough for the surgery and can be associated with intravenous premedication to ease pain [3].

Primary treatment is surgical. The first priority in treat-

ment is always to save the life of the patient. Decompression is the basic surgical principle. Even if the signs of laryngeal edema and respiratory difficulties are not present, surgical tissue decompression must be performed immediately to ease the pressure inside the tissue and the edema will not progress to the lateral areas. However, it is very important to make proper exploration for abscesses and drain them. Even though imaging studies do not show an abscess, surgery is recommended [5,23].

Surgical treatment has two purposes, the removal of the cause and the surgical decompression of the spaces involved [5]. Often in the initial cases of Ludwig's angina their treatment is seen as consisting in tooth extraction and administration of antibiotic treatment, without any surgical treatment [5, 6, 21, 24].

Decompression has three purposes: 1) Reducing tension in tissue and preventing spreading of edema, 2) Reducing the pressure in the tissues and improving blood circulation, 3) Draining septic material and preventing septicemia [5].

Among the advantages of surgical decompression are: 1) Reduces the pressure in the edema tissues, releasing the airways, 2) Allows drainage of purulent collections, 3) Allows obtaining pathogenic culture to determine the antibiotic susceptibility, 4) Allows irrigation with antiseptic solutions regulated at a certain interval, 5) Two submandibular incisions and one submental incision are sufficient for the incision and drainage of purulent collections, 6) For the drainage of the sublingual spaces, it is possible to pass through the mylohyoid muscle or we may perform separate endo-oral incisions, if the patient can open the mouth, 7) Only in case it is a serous or purulent phase, the key to success is surgical treatment [3]. The incision and drainage of the purulent collection are made regardless of the present purulent content [20].

The bilateral drainage of submandibular, sublingual and submental spaces is recommended. It is preferable to drain the sublingual and submental spaces separately, to prevent perforation of the mylohyoid muscle [3]. If trismus is present, the pterygomandibular space must be drained [10].

Free incisions of the skin and soft tissue opening are performed layer-by-layer dissection. A horizontal incision to the menton and to the hyoid bone is a classic approach to the surgical treatment of phlegmons of the mouth floor, but the collar-shape incisions are considered to be unaesthetic and less necessary. Two incisions are performed in the right and left submandibular region and one on the median line. This technique combined with sublingual space drainage prevents asphyxia and the progress of edema. The mylohyoid muscle must be dissected and penetrated into the sublingual space. A drain must be applied from the median line to the hyoid bone, at the root of the tongue. Generally, small purulent collections are obtained. Sometimes the purulent collections appear in the late phase. Sometimes a needle is used to suck the collections in order to avoid surgical procedures. This technique is not recommended because this pathology develops rapidly [10].

A surgical approach to phlegmon of the mouth floor includes a generous horizontal incision approximately 1 cm above the hyoid bone. These incisions can be extended to the submandibular gland space, with the incision of the capsule if an abscess is suspected. The platisma muscle is divided horizontally, while on the superficial layer of the deep cervical fascia, a vertical incision is made in the middle of the mandibular symphysis and the hyoid bone. Digastric muscle, mylohyoid muscle, and the variable portion of the tongue muscle are split into the middle sagittal plane to decompress the oral floor. The dissection as the size of a finger between muscles in a lateral directive is helpful in identifying and draining any abscesses. Iodized cotton wools are placed and the wound is left open [23].

Some sources claim that the incision should be made in the form of a collar [47,52]. In ancient literature, an incision shaped as a horseshoe is described in the submandibular area along the lower edge of the mandible. Two separated bilateral incisions in the submandibular regions and one in the submental area is the most practiced surgical treatment. The decompression of sublingual space is the most important thing we have gained through penetrating through the mylohyoid muscle in the sublingual space. The sublingual space can be decompressed by making an incision in the oral floor parallel to the lingual vestibule. The incision is made for decompression, in order to prevent the spread of the purulent collection and asphyxia. As the condition is non-suppurative, there is no pus. In some cases there may be located some small spaces of pus which are covered by a range of inflamed tissues. After making the incision, a corrugated rubber drain can be left in space to drain the exudate. Edema gradually decreases [5,13].

In the unilateral phlegmon of the mouth floor it is recommended to make an incision in the submandibular region of the affected part [44,41].

The medication should be initiated while the operating room is being prepared. This must include the resuscitation of the fluid and the initiation of a broad spectrum antibiotic. Infusions are necessary because patients are dehydrated. Taking patients not in time in the operating room is associated with a worse outcome [29].

The drained fluid and the pus must be sent for bacteriological investigation. The culture of the blood is usually not positive, but it can show the causal microorganism [21].

Antibiotic therapy plays a leading role in the treatment of oral phlegmon and has to be carried out both on aerobic microbial and oral anaerobic flora. Therefore, a penicillin-derived inhibitory combination such as ampicillin will be welcomed [12].

The culture of microorganisms must be detected from the operative plague as early as possible to ensure the most qualitative antibiotic treatment. Antibiotics in the form of pills can be given until the patient makes a fever, or at most 48 hours from the time of the fever. Early diagnosis and aggressive treatment with antibiotics reduced mortality to less than 2% [15].

Other initial antibiotic regimes include: clindamycin, gram negative, cephalosporins of the 3rdand 4th generation with metronidazole. In addition, if aureus streptococcus is resistant to methicillin it is a concern and can be replaced with vancomycin [12,13].

Usually, the following antibiotics are suggested: penicillin, semisynthetic derivatives of penicillin, erythromycin, cloxacillin, gentamicin, clindamycin, metronidazole [4,5,7].

The antibiotic should be changed after each test of antibiotic sensitivity. The treatment should be changed every 48 to 72 hours if the condition does not improve after treatment [3]. Corticosteroids lead to more rapid penetration of antibiotics and respiratory protection. Most often intravenous corticosteroids – dexamethasone are introduced [16].

The intravenous fluid is administered to maintain blood pressure, electrolyte balance and to provide nutrition for the patient experiencing difficulty in swallowing. Other symptomatic treatments such as analgesics and anti-inflammatory drugs may be prescribed [5,22].

Recommendations after the surgery:

1. Extubation after the edema is regressed.
2. Periodic irrigation of drains.
3. Periodic sensitivity of microorganisms to antibiotics.
4. Periodic reevaluation of blood analysis.
5. Monitoring the course of infection [7].

Local wound care is done through dressings. The drainage should be maintained until the edema regresses substantially and the purulent discharges substantially decrease or disappear [5,29].

Postoperative lavage is especially recommended for severe infections [29]. The patient should be ensured with a diet high in protein and vitamins [16].

Conclusions

1. The phlegmon of the mouth floor may involve only the unilateral spaces of the mouth, and the diffuse phlegmon of the mouth floor is bilaterally binding and is also called Ludwig's angina.
2. Patient's intubation is the elective method when it is possible.
3. Aggressive antibiotic treatment is needed as early as possible.
4. Surgical treatment is required to be performed as early as possible.
5. The number of incisions and their location is made depending on the situation.

References

1. Fleisher Lee A. Anesthesia and uncommon diseases. 6th ed. London: Elsevier Health Sciences; 2012. 664 p.
2. Eversole Lewis R. Clinical outline of oral pathology: diagnosis and treatment. 4th ed. Shelton: PMPH-SUA; 2011. 754 p.
3. Balaji SM. Textbook of oral and maxillofacial surgery. New Delhi (India): Elsevier; 2009. 707 p.
4. Swapan Kumar Purkait. Essentials of oral pathology. New Delhi (India): Jaypee Brothers Medical Publishers; 2011. 644 p.
5. Borle Rajiv M, editor. Textbook of oral and maxillofacial surgery. New Delhi (India): Jaypee Brothers Medical Publishers; 2014. 830 p.
6. Nunes RH, Abello AL, Castillo M, editors. Critical findings in neuroradiology. Cham (Switzerland): Springer; 2016. 535 p.
7. Gandhi MN, Malde AD, Kudalkar AG, Karnik HS, editors. A practical approach to anesthesia for emergency surgery. New Delhi (India): Jaypee Brothers Medical Publishers; 2011. 648 p.
8. Greenberg MI, Hendrickson RG, Silverberg M, editors. Greenberg's text-atlas of emergency medicine. Philadelphia: Lippincott Williams & Wilkins; 2004. 1079 p.
9. Bailey Byron J. Atlas of head & neck surgery--otolaryngology. Philadelphia: Lippincott Williams & Wilkins; 1996. 1021 p.
10. Hupp JR, Ferneini EM, editors. Head, neck, and orofacial infections: an interdisciplinary approach. Philadelphia: Elsevier; 2015. 496 p.
11. Marx J, Hockberger R, Walls R, et al. Rosen's emergency medicine: concepts and clinical practice. St Louis: Elsevier; 2009. 2894 p.
12. Caplivski D, Scheld WM. Consultations in infectious disease: a case based approach to diagnosis and management. New York: Oxford University Press; 2012. 392 p.
13. Park A, Price R, editors. Global surgery: the essentials. Cham (Switzerland): Springer; 2017. 597 p.
14. Sahoo GC. Emergencies in otorhinolaryngology. New Delhi (India): Jaypee Brothers Medical Publishers; 2014. 194 p.
15. Joyce Joseph A. Perianesthesia patient care for uncommon diseases. St Louis: Elsevier; 2008. 448 p.
16. Ghom AG, Ghom SA. Textbook of oral medicine. New Delhi (India): Jaypee Brothers Medical Publishers; 2014. 1144 p.
17. Adams JG, Barton ED, Collings J, Deblieux PM, Gisondi MA, Nadel ES, editors. Emergency medicine. London: Elsevier Health Sciences; 2012. 1888 p.
18. Andersson L, Kahnberg K-E, Pogrel MA. Oral and maxillofacial surgery. Chicester (United Kingdom): John Wiley and Sons; 2010. 1312 p.
19. Cameron P, Jelinek G, Kelly A, Murray L, Brown AF. Adult emergency medicine. London: Elsevier Health Sciences; 2009. 1048 p.
20. Barash PG, Cullen BF, Stoelting RK, Cahalan MK, Stock MC. Clinical anesthesia. Philadelphia: Lippincott Williams & Wilkins; 2009. 1760 p.
21. Mick N, Peters JR, Egan D, Nadel E. Blueprints emergency medicine. Philadelphia: Lippincott Williams & Wilkins; 2005. 324 p.
22. Eversole LR. Clinical outline of oral pathology. Ontario (Canada): B.C. Decker Inc; 2001. 440 p.
23. Bluestone CD, Stool SE, Alper CM, et al. Pediatric otolaryngology. 4th ed. London: Elsevier Health Sciences; 2002. 1600 p.
24. Kahn JH, Magauran BG, Olshaker J, editors. Geriatric emergency medicine: principles and practice. Cambridge: Cambridge University Press; 2014. 379 p.
25. Gorbach SL, Bartlett JG, Blacklow NR. Infectious diseases. Philadelphia: Lippincott Williams & Wilkins; 2003. 2700 p.
26. Fehrenbach MJ, Herring SW. Illustrated anatomy of the head and neck. 4th ed. St. Louis: Saunders; 2011. 320 p.
27. Marcucci C, Cohen NA, Metro DG, Kirsch JR, editors. Avoiding common anesthesia errors. Philadelphia: Lippincott Williams & Wilkins; 2007. 704 p.
28. Neville BW, Damm DD, Allen CM, Chi AC. Oral and maxillofacial pathology. London: Elsevier Health Sciences; 2015. 928 p.
29. Bagheri SC, Jo C. Clinical review of oral and maxillofacial surgery. St Louis: Mosby; 2007. 646 p.
30. Kremer MJ, Blair T. Ludwig's angina: forewarned is forearmed. AANA J. 2006;74(6):445-51.
31. Kataria G, Saxena A, Bhagat S, Singh B, Kaur M. Deep neck space infections: a study of 76 cases. Iran J Otorhinolaryngol. 2015 Jul; 27(81):293-9. 32.

32. Rowe DP, Ollapallil J. Does surgical decompression in Ludwig's angina decrease hospital length of stay? ANZ J Surg. 2011;81(3):168-71.
33. Larawin V, Naipao J, Dubey SP. Head and neck space infections. Otolaryngol Head Neck Surg. 2006;135(6):889-93.
34. Allareddy V, Rampa S, Nalliah RP, et al. Longitudinal discharge trends and outcomes after hospitalization for mouth cellulitis and Ludwig's angina. Oral Surg Oral Med Oral Pathol Oral Radiol. 2014 Nov;118(5):524-31.
35. Ramat Oyebunmi Braimah, Abdurrazaq Olanrewaju Taiwo, Adebayo Aremu Ibikunle. Ludwig's angina: analysis of 28 cases seen and managed in Sokoto, Northwest Nigeria. Saudi Surg J. 2016;4(2):77-83.
36. Vincent Ugboko, Kizito Ndukwe, Fadekemi Oginni. Ludwig's angina: an analysis of sixteen cases in a suburban Nigerian tertiary facility. African J Oral Health. 2005;2(1&2):16-23.
37. Botha A, Jacobs F, Postma C. Retrospective analysis of etiology and comorbid diseases associated with Ludwig's angina. Ann Maxillofac Surg. 2015;5(2):168-73.
38. Kulakov LA, Robustova TG, Nerobeeva AI. Khirurgicheskaia stomatologiia i cheliustno-litsevaia khirurgiia [Dental surgery. Oral and maxillofacial surgery]. Moscow: GEOTAR-Media; 2010. 928 p. Russian.
39. Bernadskii Iu, Zaslavskii I, Bernadskaia G. Gnoinaia cheliustno-litsevaia khirurgiia [Septic maxillofacial surgery]. Kiev: Zdorov'e; 1983. 242 p. Russian.
40. Poliakova V. Mestnoe lechenie gnoinyh ran litsa i shei [Local treatment of septic lesions of the face and neck]. Smolensk: [publisher unknown]; 2004. 35 p. Russian.
41. Shargorodskii A. Vospalitel'nye zabolevaniia tkanei cheliustno-litsevoi oblasti i shei [Inflammatory diseases of the tissues of maxillofacial region and neck]. Moscow: GOU VUNMT; 2001. 285 p. Russian.
42. Kravchenko V, Leshchenko I. Diagnostika i lechenie gnoinoi stomatologicheskoi infektsii [The diagnosis and treatment of dental septic infection]. Samara: Perspektiva; 2003. 248 p. Russian.
43. Timofeev A. Rukovodstvo po cheliustno-litsevoi khirurgii i khirurgicheskoi stomatologii [Guide to maxillofacial surgery and dental surgery]. Kiev: Chervona Ruta; 2002. 1022 p. Russian.
44. Ruzin G, Burykh M. Osnovy tekhnologii operatsii v khirurgicheskoi stomatologii i cheliustno-litsevoi khirurgii [Fundamentals of surgery techniques in dental and maxillofacial surgery]. Khar'kov: Znanie; 2000. 292 p. Russian.
45. Pal'chun V, Luchikhin L, Kriukov A. Vospalitel'nye zabolevaniia glotki [Inflammatory diseases of the pharynx]. Moscow: GEOTAR-Media; 2012. 288 p. Russian.
46. Trezubov V, Arutiunov S, editors. Klinicheskaia stomatologiia [Clinical dentistry]. Moscow: Medprint; 2015. 788 p. Russian.
47. Robustova TG. Khirurgichescaia stomatologiia [Surgical dentistry]. Moscow: Meditsina; 2003. 503 p. Russian.
48. Pricop M, Urtila E. Infectiile buco-maxilo-faciale [Oral and maxillofacial infections]. Timisoara: Helicon; 1994. 211 p. Romanian.
49. Ibric V, et al. Chirurgie maxilo-faciala [Oral and maxillofacial surgery]. Bucharest: Editura Eminescu; 2000. 299 p. Romanian.
50. Popescu V, Burlibasa C. Tehnici curente de chirurgie stomatologica [Current techniques of surgical dentistry]. Bucharest: [publisher unknown]; 1966. Romanian.
51. Rotaru A, Baciut G, Rotaru H. Chirurgie orala si maxilofaciala [Oral and maxillofacial surgery]. Bucharest: Editura medicala Iuliu Hatieganu; 2005. 563 p. Romanian.
52. Burlibasa C, editor. Chirurgie orala si maxilofaciala [Oral and maxillofacial surgery]. Bucharest: Editura medicala; 2005. 1312 p. Romanian.
53. Bucur A, editor. Compendiu de chirurgie oro-maxilo-faciala [Guideline of oral and maxillofacial surgery]. Bucharest: Q Med Publishing; 2009. 535 p. Romanian.

DOI: 10.5281/zenodo.1186200
UDC: [616-039.77+616-089.844]:614.2(478)

Management of the aesthetic medicine services in the Republic of Moldova

*Nedelciuc Boris[1,2], MD, PhD, Associate Professor; Lozan Oleg[1], MD, PhD, Professor;
Betiu Mircea[2], MD, PhD, Associate Professor; Gogu Vladislav[2], MD, PhD, Associate Professor;
Tabarna Vasile[2], MD, PhD, Associate Professor; Contu Ghenadie[3], MD, PhD, Associate Professor

[1]School of Public Health Management, [2]Department of Dermatovenereology, [3] Nicolae Anestiadi Department of Surgery
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

*Corresponding author: boris.nedelciuc@usmf.md. Received December 6, 2017; accepted February 12, 2018

Abstract

Background: In recent years, there has been an increasing interest in aesthetic medicine. Minor-invasive or injectable procedures, as well as laser therapy have become extremely successful. In case of any inherited or acquired, real or imaginary deformities, the invasive interventions viz. plastics and / or reconstruction are the most commonly addressed to. The aesthetic medicine in our country does not have a certain status yet. This is due to the presence of some ambiguities in the existing legal framework, as well as myths of popular perceptions. In order to assess the cosmetic and aesthetic medicine services within the instances of the Republic of Moldova, the Ministry of Health approved the establishment of a Working Group. Furthermore, based on its expertise, an Action Plan has been approved, particularly for this sector of activity, followed by the Algorithm for differentiating professional competency within the above mentioned areas. Subsequently, there have been approved steps on the organization of compliance assessment regarding the professional skills of medical staff, who work in the fields of cosmetology and dermatology. As referring to the medical staff engaged in the field of aesthetic surgery, an effective mechanism for assessing the compliance of professional skills is still going to be developed in future.

Conclusions: Based on the above-mentioned, we consider it appropriate to carry out a research for assessing the aesthetic medicine in the Republic of Moldova. The improvement and strengthening of management capabilities will further enhance the quality of medical services within these two related areas: mini-invasive aesthetic medicine (dermatology and cosmetology) and invasive aesthetic medicine (aesthetic surgery).

Key words: dermatology, cosmetology, surgery, aesthetic medicine, management.

Introduction

In the last half century, our culture has been extremely preoccupied with the image and the way we look among those who surround us. No wonder, when two candidates submit two similar CVs for a job application, the external aspect is often a decisive factor [1; 2]. What makes people believe however that the image matters that much? Is this an explanation of the role played by the artistic dimension in the development of Homo Aestheticus [3]? And what is beauty? What are its limits? Is there a standard for it? These are just a few questions that have troubled the human mind from ancient times until today [4].

Beauty is the essential virtue of the spirit and an important desire of both civilization and progress. We are the way we express ourselves: through speech, writing, appearance, attitude, and behaviour [5]. Unfortunately (or perhaps fortunately), there was not an existing universal standard in this regard and probably there is not any today or will never exist in future. Why? Because beauty is perceived within the limits of our knowledge, our education and, last but not least, within the limits of the anatomical and physiological particularities [4]. No wonder, it is said that beauty is the result of a sensory act. What one likes, another may dislike, whereas the relationship between beauty and ugliness can be reversed. This depends on the viewer [6].

An important attribute of beauty is the skin, especially the visible parts (face, neck, hands), as well as a range of ornaments, accessories (tattoos, earrings, bracelets, rings) that our ancestors used to wear, and therefore we respectively wear nowadays. Over time, millions of people have tried to change appearances and to be seen differently than they really are. This phenomenon has been recognized today as a form of hierarchical positioning in the societies of those times. Later on, various other reasons have emerged like religious, cultural, sexual etc. [4].

At a certain time, the man's preoccupation with beauty intersected (in some areas, even merged) with the medical act. As a result, towards the end of the 20th century, a new trend in medicine arose: there emerged specializations from already existing specialties [7]. Dermatology was among them. Therefore, areas such as cosmetology (science of beauty, which is a branch of dermatology studying biological aesthetics), tricology (the science on the structure, functions and diseases of the hair) and onychology (science dealing with the structure, functions and diseases of the nails) came to life.

In addition, certain interdisciplinary specializations have appeared, directly or indirectly associated with the two spheres of interest: health and beauty. These are some of them: surgical dermatology (science of invasive interventions in dermatology), oncological dermatology (science of skin tumors), photodermatology (science of dermatoses induced by photosensitization and / or photoagression), pediatric dermatology (science of infantile dermatoses), geriatric dermatology (science of senile dermatoses) etc.

Over the past 15-20 years, there has been a growing interest in aesthetic medicine. Even though, at a certain point, there were controversies regarding its ethics [1; 8; 9], the legitima-

cy of an aesthetic approach to medicine is no longer being questioned about [10]. Minor-invasive or injectable procedures (hyaluronic acid, botulinum toxin etc.) and laser therapy are being among the most successful ones [11; 12; 13].

The laser or the intelligent light was one of the most revolutionary breakthroughs of the 20th century. Due to its biological effects (analgesic, anti-inflammatory, healing, muscle relaxing, antiphlogistic, anti-edematous, antibacterial, antiviral, biostimulatory etc.), the lasers, particularly the therapeutic ones with semiconductors have been widely applied in treatment of many dermatological and cosmetic problems as acne, rosacea, herpes simplex, alopecia, hemangiomas etc. [14]. Recently, a laser related technology – intense pulsed light or IPL – has been increasingly used [13]. Unlike the laser, which uses a single wavelength (single color), the IPL technique uses a series of special filters that select different wavelengths out of the light spectrum. Targets that capture the light are called chromophores. They absorb the light, which generates heat, thus favouring the destruction of structures containing pigments, a process called selective photo-termolysis. IPL technique has many applications: hair removal, treatment of pigmented and vascular lesions, skin rejuvenation (Photo rejuvenation) etc.

Until recently, the beauty industry was largely used by women, now there is a steady increase in the number of men, as well [15]. There is also a growing number of third-age solicitors [2]. Life expectancy has increased considerably, and people have become more concerned about their appearance [16]. The phenomenon of skin aging (wrinkles, cutaneous xerosis, senile keratoses, etc.) has become a reason for more frequent visits to the dermatologist [17].

In case of inherited or acquired, real or imaginary deformities, invasive interventions are more commonly used as plasty and/or reconstruction of the nose, ears, breasts, buttocks, thighs etc. [18; 19; 20; 21].

Over the years, aesthetic surgery has passed through many syncopes and identity crises [8]. Some questions still remain unanswered today: is aesthetic surgery a medical field or a business aimed at profitable gain? There is no unanimous opinion on this. One thing is certain, however, that the field of aesthetic surgery is very dynamic, whereas new technologies and market trends dictate the relationship between supply and demand [22].

Fortunately, aesthetic medicine means not only the satisfaction of some beauty whims. Advances in plastic and reconstructive surgery have revolutionized the management of patients suffering from various congenital anomalies, burn scars, cutaneous cancers [1].

Unfortunately, the lack of competence and experience sometimes results in adverse effects and regrettable complications [23; 24; 25]. There is no considerable expertise data available on this phenomenon, since this particular sector is being marginally regulated, even in the developed countries. Therefore, a state intervention is absolutely required in order to maintain safety standards [26].

Quality management, both of medical institutions (service providers) and of educational institutions (specialist training), is another major concern [2; 27]. Thus the key to success in aesthetic medicine, as well as in many other areas, lies in an effective management of developmental programs: strategic planning, financial analysis, promotion policy, public relations, human resources, informational technology [28; 29].

The use of digital data (for clinical, educational and administrative purposes) is no longer a novelty. As a result, healthcare assistance tends to become more accessible and effective. At the same time, the huge advance of technical and scientific progress has created premises for the emergence of new approaches regarding the doctor-doctor, particularly the patient-doctor (dermatology) or client-doctor (cosmetology) relationships [30].

For a long time, aesthetic medicine was learned through apprenticeship, by attending various workshops, conferences, congresses, etc. In 1997, the European Society of Cosmetic & Aesthetic Dermatology or ESCAD was founded. Being a sister society with the European Academy of Dermatology and Venereology or EADV, ESCAD brings together the most skilful experts in the field, and offers a wide range of opportunities to the general public [16]. Under the aegis of ESCAD, the Cosmetic Medicine Journal has appeared on a quarterly basis, the English version of the Kosmetische Medizin journal, founded in 1951 and originally intended for German readers only, but which has gradually become the undisputed leader in the field of cosmetology.

On a global level, the International Academy of Cosmetic Dermatology or IACD, under the auspices of the Journal of Cosmetic Dermatology has appeared [16]. Regarding the community of aesthetic surgeons, there is the International Society of Aesthetic Plastic Surgery (ISAPS) and the International Confederation of Plastic, Reconstructive and Aesthetic Surgery (IPRAS).

Regarding our country, the Society of Dermatology in the Republic of Moldova (the legal successor of the Dermato-Cosmed Association, founded in 1994) and the Association of Plastic, Reconstructive and Aesthetic Surgery of the Republic of Moldova were established. Additionally, in 2015 a new medical project was launched – the Health & Beauty Online Journal [31]. The journal presents a modern, interactive and friendly design and is aimed at the general public, patients of all ages, and those who are already learning or working in the field among them students, residents, doctors. Our readers have access to the most cutting-edge data on skin diseases – summaries, articles and clinical cases. Moreover, it includes topics related to aesthetic, sexual and reproductive medicine issues such as interviews, comments, practical advice etc. Thus, in a relatively short period of time, the Health & Beauty Online Journal has become the most popular profile site in the republic. According to Google Analytics data, there have been over 35,000 unique visitors and over 93,000 views since it was launched. We believe that it is a good result for a country with only about 2.5 million inhabitants. This proves that online or distant communication is an undeniable reality and an effective means of documentation and learning within an ever-changing world [30].

Premises and opportunities

The importance of this topic has been largely discussed and repeatedly written within our works [32; 33; 34; 35]. In order to assess the cosmetic and aesthetic medicine services within the instances of the Republic of Moldova, the Ministry of Health approved the establishment of a Working Group. [36]. Furthermore, based on the Expertise of the Working Group and for the purpose of regulating the activity of dermato-cosmetology, the Ministry of Health approved the Action Plan particularly for this sector of activity [37]. Also, due to the suggestion of the Working Group and in order to regulate the activity of cosmetics, dermatology, plastic surgery, aesthetics and reconstructive microsurgery, the Ministry of Health adopted the Algorithm for the differentiation of professional skills in the above mentioned domains [38]. Subsequently, the Ministry of Health approved the steps on the organization of compliance assessment regarding the professional skills of medical staff, who work in the fields of cosmetology and dermatology. Regarding the healthcare engaged in the field of aesthetic surgery, an effective mechanism for assessing the compliance of professional skills is still going to be developed in future [39].

The subject matter is still a complex and delicate one, as cosmetics and cosmetology are closely related but different activities. Regretfully, many people confuse them, hence the erroneous interpretations of the staff who work in these sectors [33; 34; 38]. A retrospective view upon them will help us to better understand the similarities and differences between them:

A. Cosmetics

Cosmetics, in the classic sense, is a branch of the pharmaceutical industry that manufactures cosmetic products: skin care, hair and nail products [33; 40]. Furthermore, cosmetics is a non-medical field of activity, which aims at maintaining the human body by means of care and beauty methods/procedures. Medical studies are not mandatory. In the Republic of Moldova, necessary professional skills can be obtained within the vocational-technical institutions while the occupation is termed as beautician or cosmetic technician. The providing institutions are as follows: hairdressers, beauty cabinets, beauty salons, tattoo salons, SPA salons [38].

This activity field is regulated by the following legal acts and documents [38]: 1) Nomenclature of vocational training areas, specialties and qualifications for secondary and non-tertiary post-secondary of the Republic of Moldova and approved by the Government Decision in 2015: general domains 10 (services), educational domain 101 (personal services), vocational training domain 1012 (hairdressing and beauty services), code 101210 (hairdressing and cosmetics), cosmetic and hairdressing technician qualification [41]; 2) Classification of Occupations of the Republic of Moldova, approved by the order of the Ministry of Labor, Social Protection and Family Welfare in 2014: Major group 5 (services and trade workers), Major subgroup 51 (personal service workers), minor group 514 (hairdressers, cosmetic technicians and assimilated) [42]; 3) Regulation on Adult Continuing Education of the Republic of Moldova, adopted by the Government Decision in 2017 [43]; 4) Education Code of the RM, approved by the Parliament in 2014 [44].

The major professional skills obtained by a cosmetician are as following: a) hair care and hair-styling; b) nail care and beauty (manicure, pedicure); c) artistic makeup (day, evening, corrective makeup); d) extensions (eyelashes, hair, nails); e) sun tanning cabin (solar); f) procedures with water (SPA, thermal baths, plant wrapping, applications with algae and sludge etc.); g) non-medical massage (relaxation, refreshing); h) various non-medical masks (moisturizing, emollient, nutritive, soothing, tonic, clay); i) ear-piercing with the gun (for earrings); j) other forms of piercing (body, oral, genital); k) hair removal (with depilatory creams, wax, sugar); l) temporary or permanent tattoos (ornamental, sexual, religious, cultural etc.); m) other non-medical care and beauty interventions (33; 34; 38; 40; 45].

B. Cosmetology

Cosmetology is a branch of dermatology that aims at maintaining the human body by means of mini-invasive methods of beauty and treatment. Higher medical education is required. In the Republic of Moldova, Nicolae Testemitanu SUMPh provides professional training in this field, namely at Faculty of Medicine, the initial professional training at General Medicine, later on students pass to Postgraduate Training by means of Internship (up to 1995) or Residency (since 1996) at Specialty of Dermatovenereology. Medical graduates who lack internship or residency in dermatovenereology, but have a postgraduate training in other specialties may join: a) clinical Fellowship (until 2016), in Dermatology and Cosmetology specialization, or b) complementary studies in Dermatology and Cosmetology (since 2018). Licensed occupations are: aesthetic dermatologist or dermatologist-cosmetologist. Facility providing institutions are the following: medical cabinets, medical centers, hospitals, clinics [33; 34; 38].

The activity field is regulated by the following legal acts and documents [38]: 1) Nomenclature for the vocational training areas and specializations for academic staff development at higher educational institutions, cycle I, approved by the Parliament in 2005 [46]; 2) Education Code of the Republic of Moldova, approved by the Parliament in 2014: art.121 [44]; 3) Classification of occupations of the Republic of Moldova, approved by the Order of the Ministry of Labor, Social Protection and Family Welfare in 2014: major groups 2 (specialists in various fields of activity), major subgroups 22 (health specialists), minor groups 221 (doctors), basic groups (specialists in dermato-venereology) [42]; 4) Nomenclature of specialties for postgraduate education by means of Residency (Specialty of Dermato-venereology), approved by the Order of the Ministry of Health of the Republic of Moldova in 2015 [47]; 5) Regulation on Adult Continuing Education of the Republic of Moldova,[43]; 6) Regulation on the organization and functioning of the Commission to assess the professional skill compliance for the medical staff employed in the field of dermatology and cos-

metology, approved by the Order of the Ministry of Health [48], respectively by the Order of the Ministry of Health, Labor and Social Protection in 2017 [49]; 7) Complementary Curriculum for dermatology and cosmetology comprising 508 hours/credits per total (dermatology – 348 hours/credits and cosmetology – 160 hours/credits), approved at the Department of Dermato-venereology of Nicolae Testemitanu SUMPh in 2017 [50].

The most important professional skills of a dermato-cosmetologist are as follows: a) various medical massage techniques (manual, vacuum etc.); b) mechanical facial cleansing (eliminating pimples/comedones in pre-inflammatory acne); c) other types of facial cleansing (galvanic, ultrasound); d) curative masks (exfoliating, antiseboreal, anti-acne, anti-couperose etc.); e) epilation (electro-, laser, IPL); f) artificial ultraviolet treatment (PUVA-therapy); g) superficial (with lactic acid, with glycolic acid, with trichloroacetic acid etc.) and deep chemical peeling (with carbolic acid, with phenol etc.); h) superficial (microdermabrasion with aluminum oxide microcrystals) and deep mechanical peeling (electric brush, abrasive brush or diamond-peel microdermabrasion); i) cauterization (chemocauterization, diathermy cautery, electrocautery, radio cautery, laser cautery); j) cryodistruction (by means of liquid nitrogen and carbon snow); k) mesotherapy (micro-injections of homeopathic substances or vitamins in the middle layer of the skin); l) microneedling or dermaroller (collagen induction); m) fillers, injections of hyaluronic acid, injections of botulinum toxin; n) biorevitalization, skin plasmolifting; o) excision and removal of superficial skin formations (epidermis & dermis); p) skin biopsy (superficial-biopsy shave, by puncture-punch biopsy, by aspiration-fine needling, by curettage, incisional, excisional); q) non-surgical blepharoplasty (non-ablative, by plasma); r) non-surgical lipolysis (chemical, ultrasound, oscillating); s) non-surgical lifting (without incision – suspended or anchored); t) hair implant (STRIP method, FUE method); u) other mini-invasive medical and/or beauty therapies. Depending on the circumstances, some non-injectable or respectively non-invasive procedures/maneuvers can be performed/carried out by Graduates of the Medical Colleges under the strict supervision and guidance of a doctor [33; 34; 38; 40; 45].

Therefore, the qualification of «cosmetologist» can only be attributed to a specialist in medicine, and the «cosmetology» activity is admitted only when the organigram of a public or private medical institution includes vacancies for specialists in dermatology and cosmetology, with internship, fellowship and residency or, even more recently, complementary training in dermatology and cosmetology [38].

By the way, the term «course in cosmetology» used by the administration of vocational-technical schools is an inappropriate or even abusive. Short-term trainings, based on vocational schools, may result in getting the qualification of a beautician (cosmetics technician). This profession, however, does not allow access to therapeutical maneuvers / procedures, since these are the duties of the dermatologist-cosmetologist, as it was mentioned above.

As we have noticed, cosmetics means care and beauty, whereas cosmetology - beauty and treatment. The first is related to a non-medical field, and the second is a medical one, namely a branch of dermatology. Even though, recently, more and more people have been using the phrase «aesthetic dermatology», the term «cosmetology» has not been abandoned or got out of use. As a compromise, the term «dermato-cosmetology» is also used.

C. Aesthetic Surgery

Nevertheless, dermatology presents no «monopoly» on aesthetics or beauty. There is a range of other specialties, mostly derived from surgery, associated with biological aesthetics [33; 34]. Aesthetic surgery is a medical activity compartment, which aims to provide beauty and treatment services by means of invasive methods. Higher medical education is required. In the Republic of Moldova, Nicolae Testemitanu SUMPh provides professional training, at the Faculty of Medicine (initial professional training at Faculty of General Medicine) and/or Dentistry, followed by postgraduate training via internship (until 1992) and residency (since 1993). Medical graduates who lack internship or residency in surgery and dentistry, but have a postgraduate training in other specialties may join: a) clinical fellowship (until 2016), in Surgery – General Surgery, Plastic and Reconstructive Surgery, Microsurgery, Maxillo-facial Surgery, Vascular Surgery, Otorhinolaryngology, Ophthalmology, Oncology, Orthopedics and Traumatology, Combustiology or b) complementary studies for gaining professional skills in invasive aesthetic medicine (the Regulation is still under process). Licensed occupations are: aesthetic surgeon or plastic surgeon. Facility providing institutions are the following: medical centers, hospitals, clinics [38].

The activity field is regulated by the following legal acts and documents [38]: 1) Nomenclature for vocational training areas and specializations for academic staff development within higher educational institutions, cycle I, approved by the Parliament in 2005 [46]; 2) Education Code of the Republic of Moldova, approved by the Parliament in 2014: art.121 [44]; 3) Classification of occupations of the Republic of Moldova, approved by the Order of the Ministry of Labor, Social Protection and Family Welfare in 2014: major groups 2 (specialists in various activity fields), major subgroups 22 (health specialists), minor groups 221 (doctors) , basic groups (specialist physicians, surgical profile) [42]; 4) Nomenclature of specialties for postgraduate education by means of residency (surgical profile), approved by the Order of the Ministry of Health of the Republic of Moldova in 2015 [47]; 5) Regulation on Adult Continuing Education of the Republic of Moldova, approved by the Government Decision in 2017 [43].

The most important professional skills of the aesthetic surgeon are as following: a) surgical interventions performed on facial and scalp regions: facial lifting (Rhytidectomy, SMAS facelifting, subperiosteal lifting, temporal and frontal lifting, endoscopic lifting), blepharoplasty, rhinoplasty, otoplasty, mentoplasty, scalp reduction, flap technique, hair transplant (autografts, isografts, synthetic hair); b) mammary gland modeling operations: mammary

augmentation, breast reduction, mastopexy, mammary reconstruction, mastectomy for gynecomastia, prophylactic mastectomy (under the mamologist supervision); c) body modeling operations: abdomenoplasty, liposuction, thigh lifting, lower body lift, brahioplastia, torsoplasty, buttock augmentation, calf augmentation; d) primary varicose veins surgery: flebectomy, varicose sclerosis; e) surgical scar correction; f) skin transplantation (skin grafting, autografs, allografs etc.); g) intimate aesthetic surgery (hymenoplasty, faloplasty etc.); h) excision and removal of deep cutaneous formations (hypoderm, muscle tissue etc.); i) reconstruction of fingers (sindactilia, Dupuytren's disease); j) other plastic and / or beauty interventions [33; 34; 38].

Final Considerations

The above discussions demonstrate that aesthetic medicine is not a myth, but a reality, which is a vast, profitable and attractive interdisciplinary field for both dermatologists and other specialists. [2] Unfortunately, sometimes, cosmetologists with no higher medical education can be met or with higher medical degree, but without postgraduate medical studies and no evidence of activity in the very field. The Commission for assessing the professional skill compliance for healthcare professionals working in the field of dermato-cosmetology proves that fact. According to it, 91 cases were submitted to the expertise in 2017 [51].

The certification of the applicants was carried out in accordance with the criteria approved by the Order no.129 P§5 of 02.11.2017 regarding the completion of Annex 1 to the Order no.608 dated from 20.07.2017 on the Regulation for the organization and functioning of the Commission for assessing the professional skill compliance for healthcare professionals working in the field of dermatology and cosmetology. Thus, according to article No 11.1 of this annex, it has been decided:

a) implicit certificates – 50 persons, out of which 44 dermato-venereologists and 6 postgraduate doctors in the field of professional training, 0912 Medicine, who hold 300 or more of continuous medical education (CME) credits in dermatology and cosmetology;

b) complementary studies are recommended to gain skills in dermatology and cosmetology (508 hours per total) – 26 postgraduate doctors in the field of vocational training, 0912 Medicine, who hold less than 300 CME credits in dermatology;

c) studies at Residence is recommended, based on a contract or study fee, at Specialty of Dermatovenereology – 4 doctors without postgraduate medical studies with evidence-based practice in the field;

d) rejected cases – 11 persons, out of which 2 persons with no higher medical education, 3 – with higher medical education, but with no postgraduate medical studies and no evidence-based practice in the field, 6 – with higher medical and postgraduate medical studies (other than Dermatovenereology), but with no evidence-based practice in the field.

As there has been noticed, aesthetic medicine does not yet have a certain status in the health care system of the Republic of Moldova. This can be explained by some ambiguities within the existing legal framework. For these reasons and many others, including the public perception and myths of the people (cosmetician = cosmetologist), there still persists a number of problems as:

1. The existence of beauty cabinets/salons, which have the right to provide only non-medical services (care and beauty) but which however provide medical services as well (beauty and treatment) with no medical staff present;

2. The existence of aesthetic centers/clinics which employ medical staff who are: a) doctors with no Internship, Residence or Fellowship studies; b) doctors from other fields (emergency medicine, neurology, family medicine) with no skills in dermato-cosmetology; c) specialists with no higher medical education (biologists, chemists etc.);

3. The existence of aesthetic centers/clinics, where dermato-cosmetologists are not professionally assessed or those who do not present themselves to the Attestation Commission of the Ministry of Health.

Conclusions

In the context of the above-mentioned, we consider it appropriate to carry out a research in order to evaluate the aesthetic medicine services of the Republic of Moldova. Strengthening management capabilities will enhance the quality of medical services in these specific areas: mini-invasive aesthetic medicine (dermato-cosmetology) and invasive aesthetic medicine (aesthetic surgery).

References

1. Mousavi SR. The ethics of aesthetic surgery. J Cutan Aesthet Surg. 2010;3(1):38-40.
2. Beylot C. Place de la cosmétologie et de l'esthétique en dermatologie [Place of cosmetology and aesthetics in dermatology]. Les Nouvelles dermatologiques [Dermatology news]. 1998;17(4):244-248. French.
3. Dissanayake E. Homo aestheticus: where art comes from and why. Seattle: University of Washington Press; 1995. 320 p.
4. Nedelciuc B. Metamorfozele frumuseții între rafinament şi vulgaritate. [The metamorphoses of beauty between refinement and vulgarity]. In: [Proceedings of the "Gh. Năstase Days" Dermatology Conference]; 2014; Iasi (Romania). p. 46-47. Romanian.
5. Popovici C. Dialog cu frumusețea și sănătatea. [Dialogue with beauty and health]. Chisinau: Universitas; 1992. 187 p. Romanian.
6. Eco U. Istoria frumuseții. [History of beauty]. Bucharest: RAO; 2012, 440 p. Romanian.
7. Muset Gh, Nedelciuc B, Valuta A. Dermatologia incotro? sau dermatologia incotro! [Where are we coming from and where are we going to?] In: [Proceedings of the "Gh. Năstase Days" Dermatology Conference]; 2010; Iaşi (România). p. 19-20. Romanian.
8. Atiyeh BS, Rubeiz MT, Hayek SN. Aesthetic/Cosmetic Surgery and ethical challenges. Aesthetic Plast Surg. 2008;32(6):829-39.
9. Wiesing U. Ethical aspects of aesthetic medicine. In: Prendergast P, Shiffman M. Aesthetic medicine. Berlin: Springer Berlin Heidelberg; 2012. p. 7-11.
10. Edmonds A. Can medicine be aesthetic? Med Anthropol Q. 2013;27(2):233-52.
11. Goisis M, editor. Injections in aesthetic medicine: atlas of full-face and full-body treatment. Milan: Springer; 2014. 297 p.
12. De Maio M, Rzany B. Injectable fillers in aesthetic medicine. 2nd ed. Berlin, Heidelberg: Springer; 2014. 174 p.
13. Raulin C, Karsai S, editors. Laser and IPL technology in dermatology and aesthetic medicine. Heidelberg: Springer; 2011. 419 p.

14. Nedelciuc B, Dermatovenerologie eponimică, toponimică şi acronimică [Eponymic, toponymic and acronymic dermatovenerology]. Chisinau: [Central Printing House]; 2010. 210 p. Romanian.
15. De Maio M, Rzany B. The male patient in aesthetic medicine. Berlin, Heidelberg: Springer; 2009. 239 p.
16. André P, Payne CR. The origins of European Society of Cosmetic & Aesthetic Dermatology and Journal of Cosmetic Dermatology. J Cos Derm. 2002;1(1):4-7.
17. Evenou P. Gestione globale dell'invecchiamento cutaneo: rispettive indicazioni della cosmetologia e delle diverse tecniche [Global management of skin aging: respective indications of the cosmetology and of the different techniques]. EMC-Cosmetologia Medica e Medicina degli Inestetismi Cutanei. 2007;4(1):1-7. Italian.
18. Klassen A, Jenkinson C, Fitzpatrick R, Goodacre T. Patients' health related quality of life before and after aesthetic surgery. Br J Plast Surg. 1996;49(7):433-8.
19. Ching S, Thoma A, McCabe RE, Antony MM. Measuring outcomes in aesthetic surgery: a comprehensive review of the literature. Plast Reconstr Surg. 2003;111(1):469-82.
20. Moss TP, Harris DL. Psychological change after aesthetic plastic surgery: a prospective controlled outcome study. Psychol Health Med. 2009;14(5):567-72.
21. Hodgkinson DJ. Identifying the body-dysmorphic patient in aesthetic surgery. Aesthet Plast Surg. 2005;29(6):503-9.
22. Smith SP Jr, Williams EF. The delivery of aesthetic medicine: illegal or not? Facial Plast Surg Clin North Am. 2007;15(2):265-71.
23. Levy LL, Emer JJ. Complications of minimally invasive cosmetic procedures: prevention and management. J Cutan Aesthet Surg. 2012;5(2):121-32.
24. Wollina U, Goldman A, Naoum C. [Side effects in aesthetic medicine. Spectrum, management and avoidance]. Hautarzt. 2013;64(3):155-62. German.
25. Raulin C, Karsai S, Schmitt L. Complications in Laser surgery and IPL treatment. In: Raulin C, Karsai S, editors. Laser and IPL technology in dermatology and aesthetic medicine. Heidelberg: Springer; 2011. p. 333-344.
26. Boon Harold Tan K. Aesthetic medicine: a health regulator's perspective. Clin Gov. 2007;12(1):13-25.
27. Metelmann HR, Waite PD, Hammes S. Quality standards in aesthetic medicine. In: Raulin C, Karsai S, editors. Laser and IPL technology in dermatology and aesthetic medicine. Heidelberg: Springer; 2011. p. 377-381.
28. Wagener DL. Dermatology practice management assures practice development and efficiency. Semin Cutan Med Surg. 2000;19(3):170-2.
29. Kuechel MC. Aesthetic medicine: practicing for success. Sudbury: Jones and Bartlett Publishers; 2004, 382 p.
30. Nedelciuc B, Dumbraveanu I, Cardaniuc C. Tehnologiile informaționale în medicină [Information technologies in medicine]. Curierul Medical (Chisinau). 2016;59(3):27. Romanian.
31. Health & Beauty Online Journal [Internet]. Nedelciuc B, editor in chief. Chisinau: Health & Beauty Online Journal, c2018 – [cited 2018 Feb 13]. Available from: www.e-dermatologie.md. Romanian.
32. Nedelciuc B. Sindromul seboreic [Seborrhoeic syndrome] [Internet]. In: [Eu TV channel (Chisinau): TV program fom 2009 Sep 28]. [cited 2018 Jan 28]. Available from: https://www.youtube.com/watch?v=KA9clEfJaFU. Romanian.
33. Nedelciuc B, Contu Gh. Medicina estetică şi componentele ei: similitudini, diferențe, oportunități. [Aesthetic medicine and its components: similarities, differences, opportunities.]. In: [4th National Congress on Dermatovenerology and Cosmetology with international participation: Abstracts]; 2010 Sep 23-25; Chisinau; 2010. p. 55-57. Romanian.
34. Nedelciuc B, Betsiu M, Gogu V, Emets Iu. Kosmetika i kosmetologiia: grani soprikosnoveniia i otlichiia. Meditsinskie i iuridicheskie aspekty [Cosmetics and cosmetology: the boundaries of contacts and differences. Medical and legal aspects]. In: [1st Congress Euro-Asian Association of Dermatovenerologists: Abstracts]; 2011 March 25-26; Kiev (Ukraina); 2011. p. 58. Russian.
35. Nedelciuc B. [Regulation for cosmetologists] [Internet]. In: [Reality TV channel (Chisinau): TV program from 2017 April 11]. [cited 2018 Jan 28]. Available from: https://www.youtube.com/watch?v=VLC3-dBoEf8. Romanian.
36. [Order of the Ministry of Health of the Republic of Moldova no. 48d of 31.01.2017 as regards to the establishment of the working group on the evaluation of the cosmetic and aesthetic medicine service]. Romanian.
37. [Order of the Ministry of Health of the Republic of Moldova no. 477 of 15.06.2017 concerning the approval of the Action Plan for the regulation of activity in the field of dermato-cosmetology]. Romanian.
38. [Order of the Ministry of Health of the Republic of Moldova no.478 of 15.06.2017 concerning the approval of the Algorithm for the differentiation of professional competences in cosmetics, dermato-cosmetology, plastic surgery, aesthetic and reconstructive microsurgery]. [Official Monitor of the Republic of Moldova]. 2015 Jul 14;(244-251):art. no.1258, Romanian.
39. [Order of the Ministry of Health of the Republic of Moldova no.608 of 20.07.2017 regarding the organization of compliance assessment regarding the professional skills of medical staff, who work in the fields of cosmetology and dermatology]. Romanian.
40. Popovici A. Dermatofarmacie şi cosmetologie [Dermatopharmacy and cosmetology]. Bucharest: Editura medicala; 1982. 268 p. Romanian.
41. [Decision of the Government of the Republic of Moldova no. 853 of 14.12.2015. Nomenclature of vocational training areas, specialties and qualifications for secondary and non-tertiary post-secondary]. [Official Monitor of the Republic of Moldova]. 2015 Dec 18;(340-346):art. no. 957. Romanian.
42. [Order of the Ministry of Labor, Social Protection and Family Welfare of the Republic of Moldova no. 22 of 03.03.2014, Classification of Occupations of the Republic of Moldova (CORM - 006-14)]. [Official Monitor of the Republic of Moldova]. 2014 May 23;(120-126):art. no. 670. Romanian.
43. [Decision of the Government of the Republic of Moldova No. 193 of 24.03.2017, Regulation on the Adult Continuous Education]. [Official Monitor of the Republic of Moldova]. 2017 March 31;(92-102):art. no. 266. Romanian.
44. [Education Code of the Republic of Moldova no.152 of 17.07.2014]. [Official Monitor of the Republic of Moldova]. 2014 Oct 24;(319-324):art. no. 634. Romanian.
45. Hirjau V, Lupuleasa D, Dumitrescu AM. Dermocosmetologie [Dermocosmetology]. Iasi: Polirom; 1998. 206 p. Romanian.
46. [The Law of the Government of the Republic of Moldova no.142 of 07.07.2005, Nomenclature for the vocational training areas and specializations for academic staff development at higher educational institutions, cycle I]. [Official Monitor of the Republic of Moldova]. 2005 Jul 29;(101-103):art. no. 476. Romanian.
47. [Order of the Ministry of Health of the Republic of Moldova no.175, P§2, dated from 23.12.2015, Nomenclature of specialties for post-graduate education through residency studies]. [Official Monitor of the Republic of Moldova]. 2017 Jan 6;(2-8):art. no.9. Romanian.
48. [Order of the Ministry of Health of the Republic of Moldova no. 608 of 20.07.2017, Organization for the assessment of the professional skill compliance of the medical staff working in the field of dermato-cosmetology, Annex no.1 - Regulation working in the field of dermato-cosmetology]. [Official Monitor of the Republic of Moldova]. 2017 Aug 4;(277-288):art. no. 1449. Romanian.
49. [Order of the Ministry of Health, Labor and Social Protection of the Republic of Moldova no.129 P§5 of 02.11.2017 on the completion of Annex 1 to Order no. 608 of 20.07.2017 on the Regulation for the organization and functioning of the Commission for assessing the professional skill compliance of medical staff working in dermato-cosmetology]. [Official Monitor of the Republic of Moldova]. 2017 Nov 24;(411-420):art. no. 2041. Romanian.
50. [Proceedings No. 10 of the Dermatovenereology Department of Nicolae Testemitanu SUMPh, Complementary Curriculum for obtaining professional competencies in dermato-cosmetology, Chisinau, 30.11.2017]. Romanian.
51. [Proceedings No. 2 of the meeting of the Commission for assessing the compliance of the professional competencies of the medical staff working in the field of Dermato-cosmetology: Commission Report. Chisinau, 28.11.2017]. Romanian.

DOI: 10.5281/zenodo.1186202
UDC: 616.314-089.843

Implant supported restoration in single-tooth replacement

Cheptanaru Olga, MD, Assistant Professor

Pavel Godoroja Department of Dental Propaedeutics
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Corresponding author: olga.cheptanaru@usmf.md. Received December 29, 2017; accepted February 16, 2018

Abstract

Background: The implant-prosthetic restoration in single missing tooth is a viable treatment option for the functional rehabilitation of tooth loss. Several published studies show that the deadline and the type of functional loading of the implant for single missing teeth is not decisive for the survival and success rate of the implant. Although the conventional protocol is still the "gold standard", the immediate loading of dental implants in the fresh post-extraction socket of the aesthetic area has an excellent prognosis. Immediate functional loading is successfully adopted to minimize the treatment time with immediate and impact benefit on the patient's quality of life and satisfaction. The aesthetic result is not yet systematically included in the success criteria of the therapy through implant, although a trend for this is more common in recent publications, particularly in publications evaluating the implant prosthetic rehabilitation in the anterior maxilla and mandible. In order to obtain optimal aesthetic results, it is necessary to take into account the other aesthetic parameters, together with the chosen material for the fabrication of the implant-supported single crown and the type of prosthetic abutment.
Conclusions: The systematic review of the contemporary specialty literature examines the types of implantations and implant-supported restorations for single tooth replacement, the survival rates of the implant, the survival of the crown on the implant and the successful implant, incidence of biological, technical and aesthetical complications of the implants and prosthetic restorations on implants, the quality of life related to the oral health to patients with implant-prosthetic treatment.

Key words: single missing tooth, implant-prosthetic restoration, success rate, aesthetic result, complications.

Introduction

Edentulism – a pathological condition characterized by the absence of one or more teeth in the dental arch – is a major health problem regardless of societies, regions, ethnicities and social stratification, has a multitude of socio-economic and health repercussions. The edentulism may be single (missing one tooth), partial (missing a group of teeth) and total (all teeth missing). The prevalence of a missing permanent tooth is 2.8-8.0% (the third molar is excluded), varying by ethnicity and population. The single tooth loss is the most common in the posterior areas of the jaws; the most affected teeth are the second lower premolar, followed by the upper lateral incisors, the second upper premolar and lower incisors [1, 2].

Aesthetic and functional restoration is the main motivation of major importance for the treatment of teeth loss. Traditional methods of solving these situations are: removable prostheses, fixed partial prostheses on the tooth support and prosthetic restorations on dental implant support. The last two approaches aim for long-term success and the best aesthetic and functional outcomes: masticatory capacity, aesthetics and patient's satisfaction [3, 4].

The results of multiple experimental and clinical scientific researches have shown that osteointegrated implants – the most modern and indicated replacement solution for lost teeth – are a beneficial alternative to dental bridges. In the last decades, prosthetic restorations on implants have expanded a lot [3, 4]. However, the method has advantages, disadvantages and contraindications. Advantages of the implant-prosthetic complex: the adjacent teeth are not affected, the proximal spaces are maintained, no secondary caries appear at the prepared teeth, the alveolar crest bone is stabilized, the alveolar process is restored as a neoalveolar process, fixed prosthesis, physionomic prosthesis. Disadvantages of the implant-prosthetic complex: long-term, but limited result, risk of prosthetic abutment unscrewing, fracture risk (crown, prosthetic abutment, implant, screw), difficulty in restoring the interdental gingival papilla, risk of decementation, 2 surgical steps, high cost. Contraindications of the implant-prosthetic complex: deep occlusions, modified spaces, poor bone supply, diseases that contraindicate the surgery [5].

Types of implantations and implant – prosthetic restorations. The primary purpose of dental implants is to act as an anchoring element for the prosthetic restorations, replacing one or more lost teeth. A necessary condition for a successful implant-prosthetic treatment is the creation and maintenance of osteointegration, defined as a "direct contact between the mature bone and the endobone implant carrying the loading and masticatory pressure". Osteointegration is dependent on fundamental factors: biocompatibility, primary stability provided by the design and characteristics of the implant surface, indicated surgical technique and general condition. Another important factor affecting osteointegration is the time of implantation and loading of the implant [6].

From a historical point of view, the recommended implant installation protocol consists of 2 surgical steps with

the implant placement after a healing period without mechanical loading of 3 months for the mandible and 6 months for the maxilla. However, with increasing requirements for less invasive and rapid procedures that optimize the aesthetic requirements, a single surgical step with immediate prosthetic loading was adopted. Immediate loading can be defined as installing the crown on the implant within one week after surgery. The installation of single implants can also be classified as functional or non-functional, depending on the occlusal contact with the antagonist teeth [7].

For the treatment of single missing tooth, the implant can be installed immediately or post-extraction (immediately or within 48-72 hours after extraction), early or immediately-delayed (4-8 weeks after extraction), delayed or late, or in the formed alveolar process (4 to 6 months after extraction) [8, 9]. Implant-prosthetic restoration may be temporary (provisional) or permanent. Functional implant loading can be immediate (1 week after implant placement), early (between 1 week and 2 months after implant placement) and delayed or conventional (2 months after implant placement) [10, 11, 12, 27]. The prosthesis restoration procedure can be positioned in occlusion (immediate functional loading) or inoclussion (non-functional loading). The difference between these two procedures is the force exerted on the implant by the fixed prosthetic restoration [8].

A Cochrane review of the literature, which included 26 controlled randomized clinical studies with a total of 1217 participants and 2120 implants, assessed whether the loading period of the implant had relevant clinical implications, because the treatment period could be drastically reduced to the patient's benefit. The authors assessed the effects of immediate functional loading (1) (during 1 week after implant placement), early (between 1 to 2 months after the implant placement) and conventional (2 months after implant placement) of osteointegrated implants; (2) immediate occlusal loading as against the non-occlusal loading and early occlusal loading as against the non-occlusal loading; (3) direct loading as against the immediate, early, and conventional progressive loading. Overall, there was no convincing evidence of a clinically significant difference in prosthesis deficiency, implant failure or bone loss associated with different loading times for implants. However, the findings should be treated with caution because of the low quality of evidence [27].

Systematic reviews of the literature, controlled randomized clinical studies and comparative studies (transversal, cohort, prospective) that evaluated different implant installation procedures and prosthetic restorations for the treatment of single missing tooth in the aesthetic (anterior) or posterior area did not find any statistically significant difference in the survival and success (tissue integration, bone resorption, frequency of implant losses and biological or technical complications), different periods of installation and restoration of the implant, including deadlines and type of functional loading. Thus, there is insufficient evidence to determine the possible advantages and disadvantages or to recommend the immediate approach, the early approach or delayed approach of implant installation and restoration [8, 9, 12]. However, some authors have mentioned that implants installed immediately and early may be at a higher risk of implant failure and complications development than delayed implants, while the aesthetic result may be better at immediate implant installation after dental extraction. In addition, the immediate restoration procedure is possibly more promising in terms of healing time and costs and is associated with a higher rate of patient's satisfaction [11, 12].

Thirty years of research of ceramics on zirconium has led to significant improvements in the biomedical field, particularly in dental implantology. Now, zirconium is used not only as a veneering material, but also for the manufacture of dental implants that have improved the aesthetics and functionality for patients. Zirconium oxide dental implants, due to excellent mechanical properties, good biocompatibility and aesthetically acceptable color, have emerged as an attractive non-metallic alternative for titanium implants. Experimental studies on animals have found excellent bone-implant contact, but not under functional loading conditions [40]. The use of zirconium dioxide abutments (materials of crown support) compared to titanium abutments showed a better aesthetic result, although the differences were not statistically significant. However, more technical complications have been noted in the use of zirconium oxide abutments [41, 42].

Cement-retained single-implant restorations have become a major pillar, but there is a renewed trend towards the use of screw-retained crowns on implants. Current evidence indicates that screw-retained single-implant restorations deserve greater attention and offer clear benefits in many cases. A review of the specialty literature has revealed the following advantages of the screw-retained single-implant crowns compared to the cement-retained single-implant crowns:

1. Predictable retention and retrievability.
2. No potential for biological consequences associated with residual cement.
3. As with cement-retained restoration, the choice between metal ceramics or all ceramics.
4. Only one margin at the implant / abutment interface.
5. A single abutment / crown ceramic margin that can extend gingivally to the implant interface.
6. Nearly imperceptible blend of a composite resin in the ceramic abutment access openings.
7. One component instead of two, which may simplify the restorative process [42].

Innovations in implant and ceramic technologies now give screw-retained prostheses the potential for esthetic, functional and biological outcomes that are comparable to those for cement-retained prostheses, while providing the advantages of predictable retrievability and avoidance of residual cement. Partial filling of the abutment access channel with the support screw can help reduce the extrusion of the excess of cement in peri-implantary tissue. Angled

implants, however, remain a major indication for prostheses [42, 43].

A meta-analysis of studies published between 1995 and 2015 showed a less marginal bone mass loss and a greater survival for the cement-retained implant prostheses, but the differences were small and could not be clinically significant [44].

Survival rates of the implant and crown. In order to make the right decision about treatment, it is important to know survival rates and the incidence of technical and biological complications not only for implants but also for prosthetic works. The implant survival rate has always been the main criterion for the success of any implant-based restoration procedure, but in recent years the implant dentistry has significantly evolved to optimize the aesthetic appearance, and the preservation of hard and soft peri-implant tissues is now mandatory [13, 34].

In order to evaluate the clinical outcomes of current implant systems, the following success criteria are used: implants inserted must be immobile at the clinical examination (clinical stability), functional and without any discomfort, without suppuration, infections, peri-implant osteolysis zones on the X-ray and fracture of the implant. The loss of marginal bone mass in the first year after the application of the prosthesis should be less than 1.5 mm. After one year of functional loading of the implant, the vertical loss of bone mass should be less than 0.2 mm per year, irreversible and / or persistent signs or symptoms of pain (at palpation, percussion or function), infection, neuropathies, paraesthesia or perforations of the mandibular canal must be missing. At the end of the 5-year and 10-year observation periods, the success rate must reach 85% and 80% respectively [14].

The success of the implant with immediate functional loading is defined according to the criteria proposed by Buser and modified by Albrektsson: (1) absence of persistent pain, dysaesthesia or paraesthesia in the implant area; (2) absence of peri-implant infection with or without suppuration; (3) absence of perceptible implant mobility and (4) persistent absence of peri-implant bone resorption greater than 1.5 mm in the first year of loading and 0.2 mm annually in the following years [15].

Implant failure criteria are due to the following factors: periodontal disease, smoking, systemic diseases, infections, aging, short implants, inadequate implants, number of implants, lack or insufficiency of integration with hard and soft tissues, inappropriate prosthetic design [16].

Dental implants can be classified according to several parameters: size, material used for manufacturing, type of implant-abutment connection and treatment steps, the latter being the most frequent. A review of the Cochrane literature identified 40 controlled randomized clinical studies and compared 18 types of different implants with a follow-up period of 1 to 5 years. The authors concluded that there is no evidence that any type of dental implant has a higher long-term success rate compared to another type [17].

Ignoring the different clinical situations, the types of implant and implant restoration, cumulative survival rates of single tooth implants, reported in the specialty literature, are within 90.7-100% after one year of follow-up [11], 97.6 % (including 99.4% for the implants placement in healed alveolar ridges and 95.6% for the implants placement in post-extraction sockets, $p = 0.004$) for the first year of functional loading [18], 99.0% for the implants installed in the healed alveolar ridges and immediate prosthesis (temporary crown installed on a temporary titanium abutment and functionally loaded 24 hours after surgery, final prosthesis was functionally loaded within 6 months after the implant placement) after 1 year of functional loading [19], 96.9-97.86% for the first year of functional loading of dental implants installed transgingival, regardless of the type of restoration - immediate or delayed [11, 12], 97.6-98.4% at 2 years [8, 11], 96.1% at 3 years [20] and 95.6% at 5 years [21] for conventional loading and immediate functional loading, 96.2-100% at 5 years for implantation and conventional functional loading with different neck designs [22], 98% at 5 years of implants installed in the healed alveolar ridges (up to 3 months after dental extraction) or in post-extraction sockets of the anterior maxilla with immediate functional loading [23], 95.7% at 5 years, and 95.7% at 10 years for immediate placement of implant with immediate functional loading [24].

The obtained outcomes were comparable to single tooth rehabilitation data using conventional long-term functional loading. Three previous meta-analyses reported survival rates of implants estimated from 94.5% to 97.2% at 5 years and from 89.4% to 95.2% at 10 years for the single tooth replacement. These results suggest that immediate function in rehabilitation of a single tooth is long-term predictable, after short-term confirmation in a meta-analysis of the absence of survival difference or marginal bone resorption between conventional functional loading and immediate functional loading [13, 25, 27].

Therefore, the implant-prosthetic treatment for single missing tooth was documented as a successful procedure with predictable results during 5 years and, to a limited extent, up to 10 years of follow-up. However, a follow-up period of 5-10 years is too short to get clear information on survival rates and complication rates [13, 25]. Data on the survival of dental implants over longer periods is very limited. A retrospective observational study revealed that the cumulative survival rate of the osteointegrated implant for the single tooth replacement in maxilla and mandible was 97.1% after 25 years of follow-up. There were observed differences in survival rates between implants with different types of surfaces: for maxilla single missing tooth during 15 years and 10 years, this indicator was 95.8% for processed surfaces (plane) and 98.5% for moderately rough surfaces and for the mandibular single missing tooth during 10 and 25 years - 95.1% and 97.2%, respectively. Therefore, implant-prosthetic treatment in the single tooth gap is a long-term predictable treatment with a lower failure rate for implants with moderately rough surfaces in the maxilla implant placement [26].

A systematic review of the specialty literature, published in 2016, found that immediate placement and restoration of implant in single tooth replacement in the anterior maxilla (8 different implant systems) resulted in success (97.96%) and survival rate (98,25%) higher after an average follow-up period of 31.2 months. Single permanent crowns were restored from 3 to 6 months after the implant placement [9].

A recent meta-analysis compared the implant survival to patients with immediate functional loading or conventional functional loading of implants placed in the posterior mandible. The authors did not find a statistically significant difference between the two functional loading forms (immediate or conventional) for an average monitoring of 31.2 months on the implant survival (91.7-100% and 96.6-100% respectively; p> 0.05) [7].

A systematic and recent review of the literature evaluated the efficacy of the treatment of the maxillary and/or mandibular posterior implant supported single tooth restoration. The observation period ranged from 4 to 96 months. The survival rate, success rate (marginal bone mass loss, bleeding around the implant when palpating around the implant, deep probing around the implant), and the average amount of loss of bone mass for implants with immediate functional loading and delayed functional loading were, respectively, 96.9% (from 82.64% to 100.0%), 100% and 0.85 mm (from 0.48 mm to 1.31 mm), 96.8%, 94.1% and 0.55 mm. The survival rates, success rates and average amount of loss of bone mass in the studies, which compared implant placement with immediate functional loading with delayed functional loading, were, 96.8% (from 95.7% to 100.0%). and 96.3% (from 95.5% to 100.0%), 85.8% (from 66.7% to 100.0%) and 93.3% (from 83.3% up to 100.0%), 0.57 ± 0.57 mm (from 0.41 ± 0.57 mm to 0.90 ± 0.3 mm) and 0.55 ± 0.37 mm (from 0.04 ± 0.46 mm to 0.88 ± 0.2 mm), respectively. Although the data published over the last 5-8 years show a slightly lower success rate for delayed functional loading implants, the differences in survival and success rates between implants with immediate functional loading and delayed functional loading were insignificant [14].

The authors concluded that the prognosis for the single tooth implant placement in the molar area offers a viable treatment option for the single missing tooth in the maxillary and mandibular posterior areas [14, 28]. There is no significant difference in the survival rates of immediately and delayed implants placement, although the success rates were slightly higher to patients with delayed functional loading. The rates of survival and success of the implants placement in the posterior quadrant, regardless of the loading protocol, are comparable to those of the implants placement in the previous regions [14].

Based on two meta-analyses, the survival of implants with a single crown constituted 96.8-97.2% after 5 years of function and 95.2% after 10 years of function. The survival rate of implant-supported single crown consisted of 94.5-96.3% after 5 years of function and 89.4% after 10 years of function [2, 13, 25]. The survival rate of metal-ceramic crowns (95.4%) was statistically significantly (p = 0.005) higher than the survival rate of full ceramic crowns (91.2%) [13].

A systematic and meta-analysis of the specialty literature reviewed the effectiveness of screw-abutments with internal or external implant-abutment connection for single tooth replacement. There were studied 586 implants with external connection and 1113 implants with internal connection. The estimated percentage of single-tooth implants without complications after 3 years was 97.3% for implants with external connection and 97.6% for implants with internal connection. The authors concluded that the geometry of the implant-abutment connection does not affect the incidence of loosening of the screws. However, most of the studies included were of a short duration and can not be extrapolated on long term [39].

The survival, success and stability of the implant, as well as the aesthetic results of the prosthesis on the implant support, depend on several factors: the anatomical place, the osteotomy protocol, bone quality and quantity at the implant place, the length, diameter and color of the dental implant, characteristics and texture of the dental implant surface, axial load, correct oral hygiene, keratinized gingiva width, soft tissue level, oral bone thickness [9, 16, 20, 36, 37]. A special feature of immediately restored implants is the immediate correct modeling of the peri-implant soft tissue in the healed alveolar ridges, by the correct morphological shape of the abutment and / or the cervical portion of the single temporary crown [9, 38]. It is possible to improve gingival aesthetics by coloring in pale pink the implant's neck and abutment, irrespective of the type of implant, obtaining a more red peri-implant mucous and closer to the natural gingival color [37].

Therefore, several published studies show that the deadlines and the types of implant placement and loading in single tooth gap are not decisive to the survival of the implant, and the success rate is, at least, comparable to published data for single tooth implants placed in accordance with the standard protocol in the healed alveolar ridges.

Complications of the implants and prosthetic restorations on implants. Technical complications denote the mechanical damage of the implants, implant components and / or suprastructures [13], but biological complications – the lesions of peri-implant soft tissue and the loss of marginal bone tissue [25].

The systematic reviews of the literature and the meta-analyses of the studies, described above, conclude that, although the survival rates of implants and crowns for single tooth gap are high, biological complications (loss of osteointegration, signs of mucosal inflammation, fistulas, peri-implant mucositis, ache, haemorrhage at palpation, suppuration, dehiscence of soft tissues, progressive loss of bone mass > 2 mm, occlusal disorders), technical (fractures of the components – implant, support, crown and support screw, loss of crown retention, weakening of the support screw, decementation) and aesthetics (soft tissue recession >1 mm,

an unfavorable crown, visible crown margins, absence of the papilla) are frequently encountered [13, 29, 30].

A meta-analysis of the longitudinal studies evaluated a total number of 3223 implants to patients aged between 13 and 94 years. Were analyzed the implant survival, the survival of single crowns on implant support (irrespective of the reconstruction material and crown fixation method on the implant) and the incidence of biological, technical and aesthetic complications for an average follow-up period of at least 5 years. The evaluation of the biological complications found a cumulative rate of the soft tissue complications of 7.1% and a cumulative rate of complications for implants with bone loss> 2 mm of 5.2%. Technical complications have reached a cumulative incidence of 8.8% for the loss of screw stability, 4.1% for the loss of retention, and 3.5% for the fracture of the veneered material. The cumulative rate of aesthetic complications was 7.1% [25].

A previous meta-analysis and included in the meta-analysis described above, revealed that during the 5-year observation period, the peri-implants and soft tissue complications occurred adjacent to 9.7% of single crowns and 6.3% of implants had a bone loss of > 2 mm. For this follow-up period, the cumulative incidence of implant fractures was 0.14%, the cumulative incidence of screw and support abutment weakening was 12.7% and 0.35%, respectively. For crown complications, the cumulative incidence of ceramic fractures was 4.5% [13].

The marginal bone level around an implant is an important criterion for the success of the treatment. The loss of marginal bone tissue following the implant placement poses not only a risk of implant failure, but reduces the chance of achieving an optimal aesthetic result, which in turn, can affect the patient's satisfaction. A systematic and recent review of the prospective studies, published in 2016, based on periapical radiographs found that the average loss of marginal bone tissue around the implants placed immediately or early from the initial time up to the most recent follow-up visit (between 1 and 10 years) was lower by 1.5 mm. The authors conclude that immediate or early implant placement after one tooth extraction can be a viable treatment, with long-term survival rates and conditions of the marginal bone level corresponding to those implants conventionally placed in the healed alveolar ridges [46]. A study found that the local bone loss at the immediate functional loading of the implant placed either in the post-extraction sockets or in the healed alveolar ridges, was similar. The average value of marginal bone loss was 0.267 ± 0.161 mm during 1 year, 0.265 ± 0.171 mm during 3 years and 0.213 ± 0.185 mm during 5 years after the implant placement in the extraction sockets, 0.266±0.176 mm during 1 year, 0.219 ± 0.175 mm during 3 years and 0.194 ± 0.172 mm during 5 years after the implant was placed in the healed alveolar ridge. The results of this study showed that there is no significant difference in bone mass loss between the two investigated groups. However, a significant reduction in marginal bone loss was more pronounced in implants placed in the healed alveolar ridges ($p < 0.041$), compared to implants installed in fresh extraction sockets ($p < 0.54$) [47].

A systematic review of the literature, which evaluated the clinical result of the immediate implant placement in the aesthetic area, revealed that the deadlines and the type of functional loading (immediate, early, delayed) were not associated with the implant survival results, which allow for immediate functional loading after the immediate implant placement. Regarding the risk factors for marginal bone level change, the immediate functional loading was associated with bone mass loss that is a clinically relevant observation. In addition, the use of a flap or conjunctive tissue graft was significantly associated with greater bone loss. Unfortunately, the few randomized clinical studies included, were based on the results of a short follow-up period (1 year). Therefore, studies with longer follow-up are required to predict the long-term aesthetic result [72, 24].

A recent study has prospectively evaluated the result at 12 years of implant-based restorations for single tooth replacement. Initially, through a two-step protocol, were placed 45 Astra Tech TiOblast ST self-tapping implants on 40 subjects with an average age of 40.9 years. The abutment connection was performed at 3 months for the mandible and at 6 months for the upper jaw after the implant placement, and the metal-ceramic prosthetic crown was fixed by cement approximately 4 weeks after connecting the abutment. At 12 years, 31 patients and 35 implants were available for evaluation. Cumulative failure was 10.3% at the subject level and 9.1% at the implant level. The total average value of bone loss was 0.67 mm at the subject level and 0.47 mm at the implant level. Nine implants (25%) showed clinical signs of inflammation, three subjects (10%) and three implants (8.6%) were diagnosed with peri-implantitis (signs of inflammation in combination with ≥2 mm bone loss radiologically assessed). Five subjects presented technical complications: 3 incidents of weakening of the abutment screw retention in the first 5 years and 2 minor ceramic fractures of the crown between 5 and 12 years of follow-up. Aesthetic complications have not been reported. The authors concluded that the use of Astra Tech dental implants is a valid treatment alternative for the single tooth replacement (48).

Based on the findings of 10 clinical studies, with a follow-up period of more than 5 years, a systematic review of the literature estimated an average failure rate of 4.8% of implant-supported restorations for single tooth replacement [25]. However, long-term studies (10 years and more) that document failures and various types of complications of replacement procedures for a missing tooth are insufficient [49]. In a systematic review of the literature, was described the incidence of biological and biomechanical complications associated with the use of implants to replace a single tooth. The authors concluded that while all prospective longitudinal studies (over 5 years) included reports on the frequency of implant loss, only a limited number of publications have presented conclusions related to the biological and technical complications. Because the prevalence of

complications may increase with the implant function time, there is a clear need for long-term data (10 years and longer) from prospective studies of implant-supported restorations for single tooth replacement [50].

A study, which evaluated the results for a follow-up period of more than 10 years for the Astra Tech single dental implants placed in the anterior maxilla, reported an average loss of marginal bone of 0.75 mm, a subject (5%) with bone loss > 2 mm and three subjects (15%) with bone loss between 1 and 1.4 mm, findings comparable to the previous study [51].

Another study assessed 40 subjects and 44 implants (Brånemark) up to 18 years. The average value of marginal bone loss from the moment of crown placement was 0.4 mm. A recent publication, which evaluated the survival rate after 10 years of function of 86 implants (Biomet 3i) with immediate restoration for single missing tooth in the anterior maxilla, found an average bone loss of 1.01 mm [52].

Several long-term prospective studies (10 years and more) reported an incidence of technical complications varying between 7% and 30% [48], 2 recimented crowns (10%), 2 minor fractures of ceramic crown (10 %) and weakening the capacity of retention of the abutment screw in 2 cases (10%) [51], 2 (3%) patients with weakening of the retention screw of the abutment and 3 (4%) patients with minor fractures of the ceramic crown [83].

Following the immediate implant placement, the recession of the peri-implant mucosa > 1 mm occurs in 9-41% of cases between 1 and 3 years, whereas early implant placement has a very low risk for recession > 1 mm [34]. The level of the peri-implant papilla for a single tooth in the anterior maxilla is mainly influenced by the interproximal bone crest level of the adjacent tooth. The marginal mucosa level is affected by several factors: peri-implant biotype, facial bone crest level, implant angle, interproximal bone crest level, and implant platform depth and bone-implant primary contact level [35].

The overall cumulative rate of complications after 18 years of follow-up was 57% and 1/3 of these complications require the renewal of at least one component. Over 50% of the complications occurred during the first 5 years of the study. In total, 36% of restorations were affected by technical complications, 24% - aesthetic complications and 20% - biological adverse reactions. The study confirms an over time increase in complications. The weakening of the support screw, a previous frequent complication, was significantly reduced by the implementation of CeraOne support abutments [29].

Dental restoration is a difficult procedure, because physicians and patients have established strict success criteria. This standard has placed the focus of research on improving the results of hard and soft tissues, the aesthetics of restoration and patient's satisfaction. In order to evaluate the complete aesthetic restoration, are widely applied the "white aesthetic score" of the implant, the "pink aesthetic score", to accurately describe all the features of the adjacent soft tissues, "the aesthetic index of the implant crown" – objective index that measures the aesthetic result of the implant crown for one single tooth (shape, color, superficial characteristics) and of the related mucosa. These indicators became popular and a standard tool for assessing the aesthetic result of implant restorations in the anterior aesthetic area, determined mainly by the shape of peri-implant pink soft tissues, the contour and color of the permanent crown [12, 19, 23].

However, a review of the international specialty literature has revealed that although there seems to be a growing interest in aesthetics in implant therapy, there are still no well-defined and universally accepted assessment criteria for aesthetic outcomes. A wide variety of parameters, methods, units of measurement, and time points used to evaluate the aesthetics, were found in different studies, which influences the validity and requires prudent interpretation of the results in comparison of the studies. Generally, until 1990, researchers were primarily interested in the functional aspects of the implant therapy; the evaluations of aesthetic results among the criteria for success only appear in more recent studies and refer mainly to the rehabilitation of the implants in the anterior maxilla area. It is obvious that the peri-implant mucosa has a significant influence on the aesthetic outcome of the therapy with dental implants [45].

The replacement of the affected teeth of the patient with immediate fixed implant-supported prostheses with functional loading represents a major achievement in the reconstructive dentistry. In recent years, the implant dentistry has increasingly supported the concept of immediate functional loading, even in the restoration of a single implant. This operative procedure is a viable and safe treatment option for both the maxilla and the mandible and has several advantages for the patient. Firstly, it eliminates the removable prosthesis, and secondly, it allows that most work times to be concentrated during a single visit, due to the computer assisted surgery. Many authors have pointed out that immediate functional loading protocols allow for better aesthetic results compared to delayed functional loading, especially in post-extraction implant placement, are able to maintain the original aesthetics of soft tissues. The restriction of bone remodeling, reduction of procedures number, shortening of the treatment period, rapid restoration of function and aesthetics contribute to the shortening of the recovery period with functional, cosmetic and psychological benefits, and reducing discomfort for patients [6, 12, 14, 18, 30].

Several comparative studies (transversal, cohort, prospective), based on the preliminary clinical and radiographic results, found that after 2 years, about 26% of cases were esthetic failures (pink aesthetic score <8 and / or white aesthetic score <6), 13% of cases showed almost perfect aesthetic results (pink aesthetic score ≥12 and / or white aesthetic score ≥9) and 61% of cases showed acceptable aesthetic results. Although the rate of aesthetic failure is quite high, the indicator falls within the range of specialized publications (5-34%) [10, 31, 32]. Thus, the early and conventional implant-prosthetic treatment of the single missing

tooth has comparable aesthetic results. The result is considered satisfactory if the pink aesthetic score is ≥8 and perfect if the pink aesthetic score is ≥12 [10].

Oral health-related quality of life in patients with implant-prosthetic restorations. In the field of implant dentistry, there is an increasing emphasis on the results reported by the patient. Patient's satisfaction is also essential for achieving this goal and can truly indicate the success of implant-prosthetic treatment from the patient's perspective [12, 19, 23]. The quality of life related to the oral health is defined as the effect of deficiencies, disabilities or handicap from an oral condition to frequent daily activities (masticatory function, speech, tooth cleaning, sleep, smile and social contact) [33].

Several prospective randomized clinical studies have comparatively evaluated the patient's satisfaction based on the Oral Health Impact Profile (OHIP-14) questionnaire on immediate functional loading and delayed functional loading of dental implants for single tooth replacement in the anterior maxilla. After 12 months, has been found a statistically significant overall improvement in the quality of life related to the oral health for both groups. Significant improvement is, probably, a result of increased comfort during the ingestion of the food, and a lower sense of insecurity and embarrassment [6, 19].

The OHIP-14 score for patients with implants placement in the healed alveolar ridges or fresh extraction sockets decreased from 0.50 at baseline to 0.17 at 6 months of follow-up ($p < 0.001$) - an improvement in all the aspects. For both groups, the score remained stable for up to 5 years ($p = 0.41$). However, after 5 years, the OHIP-14 total score showed a statistically significant greater improvement in the group with implants placed in the healed alveolar ridges, compared to the group with implants placed in the extraction sockets ($p = 0.027$) [23].

Conclusions

1. The implant-prosthetic restoration in single tooth replacement is a viable treatment option for functional restoration of tooth loss. Several published studies show that the time and the type of implant functional loading of single tooth replacement are not decisive to the survival and success rate of the implant.
2. Although the conventional protocol is still the "gold standard", immediate restoration of the implants placed in the post-extraction sockets of the aesthetic area has an excellent prognosis. Immediate functional loading is successfully adopted to minimize the treatment time with immediate benefit and relevant impact on the patient's quality of life and satisfaction.
3. The aesthetic outcome is not yet systematically included in the success criteria of implant therapy, although a trend for this is more frequent in recent publications, particularly in publications evaluating the implant-supported single tooth restorations in the anterior maxilla and mandible.
4. The chosen reconstruction material and the type of abutment for manufacturing the implant restoration do not ensure aesthetic results, if all parameters are not taken into account.

References

1. Edelmayer M, Woletz K, Ulm C, et al. Patient information on treatment alternatives for missing single tooth - Systematic review. Eur J Oral Implantol. 2016;9 Suppl 1:S45-57.
2. Friberg B. Bone augmentation for single tooth implants: A review of the literature. Eur J Oral Implantol. 2016;9 Suppl 1:S123-34.
3. Karl M. Outcome of bonded vs all-ceramic and metal- ceramic fixed prostheses for single tooth replacement. Eur J Oral Implantol. 2016;9 Suppl 1:S25-44.
4. Sghaireen MG, Al-Omiri MK. Relationship between impact of maxillary anterior fixed prosthodontic rehabilitation on daily living, satisfaction, and personality profiles. J Prosthet Dent. 2016;115(2):170-6.
5. Dierens M, Vandeweghe S, Kisch J, et al. Cost estimation of single-implant treatment in the periodontally healthy patient after 16-22 years of follow-up. Clin Oral Implants Res. 2015;26(11):1288-96.
6. Gjelvold B, Kisch J, Chrcanovic B, et al. Clinical and radiographic outcome following immediate loading and delayed loading of single-tooth implants: Randomized clinical trial. Clin Implant Dent Relat Res. 2017;19(3):549-58.
7. Moraschini V, Porto Barboza E. Immediate versus conventional loaded single implants in the posterior mandible: a meta-analysis of randomized controlled trials. Int J Oral Maxillofac Surg. 2016;45(1):85-92.
8. Mangano C, Raes F, Lenzi C, et al. Immediate loading of single implants: a 2-year prospective multicenter study. Int J Periodontics Restorative Dent. 2017;37(1):69-78.
9. Weigl P, Strangio A. The impact of immediately placed and restored single-tooth implants on hard and soft tissues in the anterior maxilla. Eur J Oral Implantol. 2016;9 Suppl 1:S89-106.
10. Cosyn J, Eghbali A, De Bruyn H, et al. Single implant treatment in healing versus healed sites of the anterior maxilla: an aesthetic evaluation. Clin Implant Dent Relat Res. 2012;14(4):517-26.
11. Barone A, Toti P, Quaranta A, et al. The clinical outcomes of immediate versus delayed restoration procedures on immediate implants: a comparative cohort study for single-tooth replacement. Clin Implant Dent Relat Res. 2015;17(6):1114-26.
12. Slagter KW. Immediate dental implant placement in the aesthetic zone [thesis]. Drukkerij van der Eems, Heerenveen, Netherlands: University Medical Center Groningen; 2016. 156 p.
13. Jung R, Pjetursson B, Glauser R, et al. A systematic review of the 5-year survival and complication rates of implant-supported single crowns. Clin Oral Implants Res. 2008;19(2):119-30.
14. Moy P, Nishimura G, Pozzi A, et al. Single implants in dorsal areas - A systematic review. Eur J Oral Implantol. 2016;9 Suppl 1:S163-72.
15. Malchiodi L, Cucchi A, Ghensi P, et al. Evaluation of the esthetic results of 64 nonfunctional immediately loaded postextraction implants in the maxilla: correlation between interproximal alveolar crest and soft tissues at 3 years of follow-up. Clin Implant Dent Relat Res. 2013;15(1):130-42.
16. Vozza I, Barone A, Quaranta M, et al. A comparison between endodontics and implantology: an 8-year retrospective study. Clin Implant Dent Relat Res. 2013;15(1):29-36.
17. Esposito M, Ardebili Y, Worthington H. Interventions for replacing missing teeth: different types of dental implants. Cochrane Database Syst Rev. 2014;(7):CD003815.
18. Del Fabbro M, Ceresoli V, Taschieri S, et al. Immediate loading of postextraction implants in the esthetic area: systematic review of the literature. Clin Implant Dent Relat Res. 2015;17(1):52-70.
19. Fügl A, Zechner W, Pozzi A, et al. An open prospective single cohort multicenter study evaluating the novel, tapered, conical connection implants supporting single crowns in the anterior and premolar maxilla: interim 1-year results. Clin Oral Investig. 2017;21(6):2133-42.
20. Guarnieri R, Grande M, Ippoliti S, et al. Influence of a Laser-Lok surface on immediate functional loading of implants in single-tooth

replacement: three-year results of a prospective randomized clinical study on soft tissue response and esthetics. Int J Periodontics Restorative Dent. 2015;35(6):865-75.
21. Donati M, La Scala V, Di Raimondo R, et al. Marginal bone preservation in single-tooth replacement: a 5-year prospective clinical multicenter study. Clin Implant Dent Relat Res. 2015;17(3):425-34.
22. den Hartog L, Meijer H, Vissink A, et al. Anterior single implants with different neck designs: 5 year results of a randomized clinical trial. Clin Implant Dent Relat Res. 2017;19(4):717-24.
23. Raes S, Raes F, Cooper L, et al. Oral health-related quality of life changes after placement of immediately loaded single implants in healed alveolar ridges or extraction sockets: a 5-year prospective follow-up study. Clin Oral Implants Res. 2017;28(6):662-7.
24. Maló P, de Araújo Nobre M, Lopes A, et al. Single-tooth rehabilitations supported by dental implants used in an immediate-provisionalization protocol: report on long-term outcome with retrospective follow-up. Clin Implant Dent Relat Res. 2015;17 Suppl 2:e511-9.
25. Jung R, Zembic A, Pjetursson B, et al. Systematic review of the survival rate and the incidence of biological, technical, and aesthetic complications of single crowns on implants reported in longitudinal studies with a mean follow-up of 5 years. Clin Oral Implants Res. 2012;23 Suppl 6:2-21.
26. Jemt T. Single-implant survival: more than 30 years of clinical experience. Int J Prosthodont. 2016;30(6):551-8.
27. Esposito M, Grusovin M, Maghaireh H, et al. Interventions for replacing missing teeth: different times for loading dental implants. Cochrane Database Syst Rev. 2013;(3):CD003878.
28. Anitua E, Murias-Freijo A, Flores J, et al. Replacement of missing posterior tooth with off-center placed single implant: Long-term follow-up outcomes. J Prosthet Dent. 2015;114(1);27-33.
29. Dierens M, De Bruyn H, Kisch J, et al. Prosthetic survival and complication rate of single implant treatment in the periodontally healthy patient after 16 to 22 years of follow-up. Clin Implant Dent Relat Res. 2016;18(1):117-28.
30. Cosyn J, Eghbali A, Hermans A, et al. A 5-year prospective study on single immediate implants in the aesthetic zone. J Clin Periodontol. 2016;43(8):702-9.
31. Meijndert L, Meijer H, Stellingsma K, et al. Evaluation of aesthetics of implant-supported single-tooth replacements using different bone augmentation procedures: a prospective randomized clinical study. Clin Oral Implants Res. 2007;18(6):715-9.
32. Belser U, Grütter L, Vailati F, et al. Outcome evaluation of early placed maxillary anterior single-tooth implants using objective esthetic criteria: a cross-sectional, retrospective study in 45 patients with a 2- to 4-year follow-up using pink and white esthetic scores. J Periodontol. 2009;80(1):140-51.
33. Angkaew C, Serichetaphongse P, Krisdapong S, et al. Oral health-related quality of life and esthetic outcome in single anterior maxillary implants. Clin Oral Implants Res. 2017;28(9):1089-96.
34. Boardman N, Darby I, Chen S. A retrospective evaluation of aesthetic outcomes for single-tooth implants in the anterior maxilla. Clin Oral Implants Res. 2016;27(4):443-51.
35. Nisapakultorn K, Suphanantachat S, Silkosessak O, et al. Factors affecting soft tissue level around anterior maxillary single-tooth implants. Clin Oral Implants Res. 2010;21(6):662-70.
36. Kamperos G, Zambara I, Petsinis V, et al. The impact of buccal bone defects and immediate placement on the esthetic outcome of maxillary anterior single-tooth implants. J Oral Implantol. 2016;42(4):337-41.
37. Gil M, Ishikawa-Nagai S, Elani H, et al. A prospective clinical trial to assess the optical efficacy of pink neck implants and pink abutments on soft tissue esthetics. J Esthet Restor Dent. 2017;29(6):409-15.
38. Schoenbaum TR, Swift EJ Jr. Abutment emergence contours for single-unit implants. J Esthet Restor Dent. 2015;27(1):1-3.
39. Lewis M, Klineberg I. Prosthodontic considerations designed to optimize outcomes for single-tooth implants. A review of the literature. Aust Dent J. 2011;56(2):181-92.
40. Siddiqi A, Khan A, Zafar S. Thirty years of translational research in zirconia dental implants: a systematic review of the literature. J Oral Implantol. 2017;43(4):314-25.
41. Carrillo de Albornoz A, Vignoletti F, Ferrantino L, et al. A randomized trial on the aesthetic outcomes of implant-supported restorations with zirconia or titanium abutments. J Clin Periodontol. 2014;41(12):1161-9.
42. Priest G. A current perspective on screw-retained single-implant restorations: a review of pertinent literature. J Esthet Restor Dent. 2017;29(3):161-71.
43. Al Amri MD, Al-Johany SS, Al-Qarni MN, et al. Influence of space size of abutment screw access channel on the amount of extruded excess cement and marginal accuracy of cement-retained single implant restorations. J Prosthet Dent. Epub 2017 May 26.
44. Lemos CA, de Souza Batista VE, Almeida DA, et al. Evaluation of cement-retained versus screw-retained implant-supported restorations for marginal bone loss: A systematic review and meta-analysis. J Prosthet Dent. 2016;115(4):419-27.
45. Annibali S, Bignozzi I, La Monaca G, et al. Usefulness of the aesthetic result as a success criterion for implant therapy: a review. Clin Implant Dent Relat Res. 2012;14(1):3-40.
46. Schropp L, Wenzel A. Timing of single implant placement and long-term observation of marginal bone levels. Eur J Oral Implantol. 2016;9 Suppl 1:S107-22.
47. Berberi AN, Sabbagh JM, Aboushelib MN, et al. A 5-year comparison of marginal bone level following immediate loading of single-tooth implants placed in healed alveolar ridges and extraction sockets in the maxilla. Front Physiol. 2014;5:29.
48. Donati M, Ekestubbe A, Lindhe J, et al. Implant-supported single-tooth restorations. A 12-year prospective study. Clin Oral Implants Res. 2016;27(10):1207-11.
49. Scheuber S, Hicklin S, Brägger U. Implants versus short-span fixed bridges: survival, complications, patients' benefits. A systematic review on economic aspects. Clin Oral Implants Res. 2012;23 Suppl 6:50-62.
50. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. J Clin Periodontol. 2002;29 Suppl 3:197-212.
51. Gotfredsen K. A 10-year prospective study of single tooth implants placed in the anterior maxilla. Clin Implant Dent Relat Res. 2012;14(1):80-7.
52. Bergenblock S, Andersson B, Fürst B, et al. Long-term follow-up of CeraOne™ single-implant restorations: an 18-year follow-up study based on a prospective patient cohort. Clin Implant Dent Relat Res. 2012;14(4):471-9.
53. Brägger U, Karoussis I, Persson R, et al. Technical and biological complications/failures with single crowns and fixed partial dentures on implants: a 10-year prospective cohort study. Clin Oral Implants Res. 2005;16(3):326-34.

BOOK REVIEW

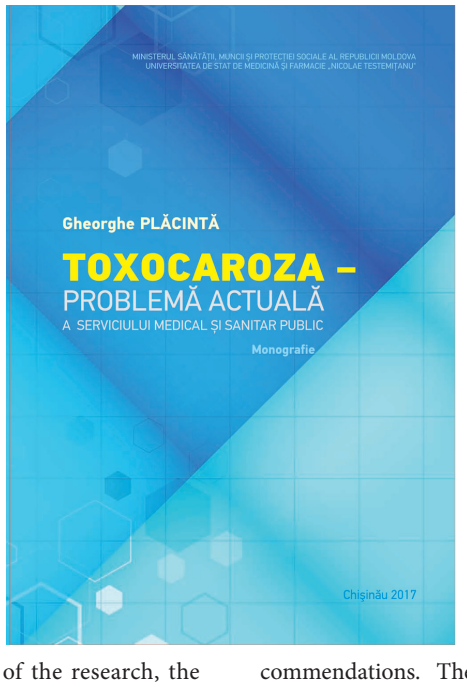
The monograph "Toxocariasis – Current Issue of the Medical and Sanitary Services"

Printing company "Tipografia Sirius", Chisinau, 2017, 240 pages

The author: **Gheorghe Placinta,** MD, PhD, Associate Professor, Department of Infectious Diseases
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

Human toxocariasis is a major medical-social problem with global spread, but still remains one of the most misunderstood parasitic invasion in humans, with complex and difficult problems of modern infectology and the entire public health and medical service.

The research and the author's own experience over 15 years in this field have made it possible to identify the factors resulting in the high degree of toxocariasis invasion; and has been developed the management algorithm.



The writing is exposed on 240 pages with affirmative material presented in 49 tables, 44 figures, 12 clinical cases, 4 annexes, and a bibliography citing 340 scientific sources.

The work begins with **Introduction** where is elucidated the actuality of the subject studied and are outlined the purpose and objectives of this research.

In the first chapter is analyzed the complex vision in the evolution of toxocariasis according to current literature. There are presented epidemiological aspects and trends in the world, the factors identifying increased degrees of infestation. The pathogenic specificity, complemented by modern explorations and multiplicity of clinical manifestations are analyzed. The need for further studies on toxocariasis and the accumulation of new data in diagnosis, therapy, and prevention are highlighted.

Chapter II describes the general design of the research, the characteristics of the patients' lots, the methods of research and statistical processing. In the study were included: 99 healthy people; 12,637 patients with various pathologies; 1166 patients toxocariasis supervised for a period from 3 to 15, 944 soil samples, and 1459 dogs' samples which have been checked to detect the presence of *T. canis* eggs, with statistical processing of the incidence of the most common helminthiasis between 2006 and 2015.

The third chapter is devoted to the identification of the factors that influence the increased toxocariasis seroprevalence in the Republic of Moldova, which is one of the highest in the world - 58.8%, with growth rate in the last 10 years of 37.2%, with prevalence of the mature individuals compared to children. The results of the study showed a strong inverse correlation ($r_{xy} = -0.785 \pm 0.017$) between the age and the proportion of seropositive results in *T. canis*. Direct correlation between peak levels of toxocariasis antibody levels, addressability and admission ($P < 0.05$) was determined.

Chapter IV reflects the magnitude and the totality of clinical manifestations in the acute, subacute and chronic form that counts over 50 clinical signs. The author has shown that in the acute evolution of toxocariasis the larvae migrans visceralis syndrome manifests only in 21.1% cases, while in 78.9% at the very onset sets occult form. The probability of healing in the acute form is only 2.3%. Children in 58.8% do not form post-vaccine immunity. The evolution differences in toxocariasis with mono-invasion and toxocariasis with different comorbidities are clear, with a clearly superior prevalence of toxocariasis injury versus associated parasitoses. Effectiveness of specific medication is demonstrated.

Chapter V establishes clinical and laboratory determinants in the different evolution of chronic toxocariasis. Thus, early cure in the first year of monitoring and timely therapy only occurs in 5.5%. Evolution by exacerbation occurs in 5.0 % of cases. All factors that contribute to exacerbation, remission, stagnant development are determined. The patho-invasive actions in the establishment of hepatic cytolysis and leukopenia and the immunodeficiency states are demonstrated.

The results in **chapter six** identify the differences in the evolution of toxocariasis established over a 10-year surveillance period. Assay indicators in time healing, serological sequelae, latent and leaps evolution are determined. It considers the significance of different treatment regimens, the utility of repeated treatments. The algorithm of conduct was developed in each case separately.

The work ends with conclusions and recommendations. The author has shown that toxocariasis presents an important medical and social problem of national interest, which requires the efforts of the whole society in diminishing the morbidity with strategic, multisectoral involvement, with a complex, lasting program. The data resulting in a very high seroprevalence, different manifestations (over 50 signs), often discrete, unexplainable, with exaggerated costs in expensive investigations, require from the very beginning the serological screening of toxocariasis for all patients hospitalized in different medical-sanitary departments in the Republic of Moldova. This will allow an early diagnosis with a considerable cost reduction.

The work fully complies with the requirements, is elaborated by a thorough study, with multifactorial analysis, with recommendations addressed to the public-health services, offering distinct management solutions for practicing physicians and doctors of different specialties, as well as to all those interested in this pathology and may be recommended for publishing.

Victor Pantea, MD, PhD, Professor
Department of Infectious Diseases
Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

GUIDE FOR AUTHORS

The authors are kindly requested to visit our web site www.moldmedjournal.md and strictly follow the directions of the **Publication Ethics and Malpractice Statement.**

The articles must be sent electronically to editor@moldmedjournal.md by the author, responsible for the correspondence, using the **Authorship Statement Form** (www.moldmedjournal/authorship-statement/.

All papers are to be executed in the following manner:

1. The manuscripts should be typed in format A4, 1.5-spaced, with 2.0 cm margins, printing type 12 Times New Roman, in Microsoft Word.

2. The title page should include the first and family name of all the authors, their academic degrees, the name of the department and institution from which the paper has arrived, the phone number and e-mail address of the corresponding author.

3. The abstract should be written on the title page and limited from 220 to 240 words. The abstract of research articles should have four parts: Background, Material and methods, Results, Conclusions. The abstract of review articles should have two parts: Background and Conclusions. The abstract should end with 3 to 6 key words.

4. The text of clinical or experimental articles (has to be less than 16 pages long) should consist of an Introduction, Material and Methods, Results, Discussion, Conclusions and be followed by not more than 40 references. **The review articles** must not exceed 25 pages and contain not more than 100 references.

5. The tables and figures must be typed, consecutively numbered and followed by an explanatory text. The figures that have to emphasize a comparison or details are published in color. If colored figures are to be placed, the author must pay an additional fee of €100 per page (1-8 figures on a page).

6. The references are to be listed in order of their appearance in the text, and the appropriate numbers are to be inserted in the text in square brackets in proper places. The references must comply with the general format outlined in the Uniform Requirements for the Manuscripts Submitted to Biomedical Journals developed by the International Committee of Medical Journal Editors (www.icmje.org), chapter IV.A.9.

7. The references in the Cyrillic script should be transliterated into Latin script using the American Library Association and Library of Congress Romanization Tables as follows: А–A, Б–B, В–V, Г–G, Д–D, Е–E, Ё–E, Ж–ZH, З–Z, И–I, Й–I, К–K, Л–L, М–M, Н–N, О–O, П–P, Р–R, С–S, Т–T, У–U, Ф–F, Х–KH, Ц–TS, Ч–CH, Ш–SH, Щ–SHCH, Ъ–", Ы–Y, Ь–', Э–E, Ю–IU, Я–IA. Immediately after the transliteration the translation of the title in English in the square brackets should follow. For example: Ivanov IV, Shchukin NF, Men'shikov VM, Ad"yunktov AM. Transplantatsiia organov i tkanei [Transplantation of organs and tissues]. Vestnik Khirurgii. 2010; 26(6):45-49. Russian.

Submission and Peer Review Process

1. Submitted articles are first put under consideration to decide whether a given article fits into the area of the journal thematic, then articles are sent to reviewers for further approval, usually to leading experts in the field. The names of the authors and reviewers and their affiliation are not shown to each other.

2. For an article to be published, it must have a well-reasoned approval of the two reviewers, which is then considered and further approved by the Editorial Board. If one of two reviews is "negative", the Editorial Board is approving the rejection or the article is sent to another reviewer.

3. If the article requires some modifications, it is returned to the author(s) to be revised, taking into account the reviewer's recommendations. The modified version is then again forwarded to the reviewer, to consider a possibility for the publication of the article.

4. The accepted article is forwarded for scientific editing and proofreading. The final version is adjusted with authors for approving and for correction of possible inexactitudes. However, essential modifications by the author(s) after editing and proofreading are not allowed.

5. In case an article is rejected, the author(s) receive a well-reasoned explanation from the Editorial Board.

Responsibility of the Editorial Board

1. The Editorial Board members are responsible for making decisions on accepting or rejecting the submitted articles. Criteria for publication are the scientific value and originality of materials and their conformity with the scope and thematic range of the journal.

3. The Editorial Board considers only previously unpublished manuscripts, containing the results of original research or detailed thematic review on the medicine, dentistry, pharmacy, social medicine and public health. In case of submitting a previously published article (or sent for publication in any other journal), repeating the pending article for more than 50%, the Editorial Board reserves the right to reject the article from being published in the "The Moldovan Medical Journal".

4. The editors do not impose authors to cite papers published in "The Moldovan Medical Journal" in order to artificially improve scientometric indicators of the journal.

5. The Editorial Board ensures that submitted material will be subject for a peer review by anonymous independent experts.

Responsibility of authors

1. Authors are responsible for the content of the article, confirming the use of only original scientific data. In case of borrowing materials from other researchers (or from an earlier publication of the given authors) the correct reference or citation should be made. Regarding plagiarism and fraudulent data please read chapter 2.3, 2.4 and 4 of the Publication Ethics and Malpractice Statement.

2. Authors guarantee that submitted article has not been previously published in English or any other language nor simultaneously submitted to another journal.

3. Authors should write the article in conformity with the Guide for Authors. Otherwise, the Editorial Board reserves the right to reject the article from consideration for publication.

Responsibility of reviewers

1. Reviewers must present an objective expertise of the manuscript. In case of any conflict of interests (self-interest, personal animosity, financial or scientific conflicts, etc), the reviewers must immediately inform the Editorial Board about it.

2. Reviewers are required to remain confidential and not to disclose information about or from the article under review to an unauthorized person.

Address of the Editorial Office

192, Stefan cel Mare Avenue, Chisinau, MD-2004, the Republic of Moldova
Telephone: +37322205209, +37322244751, +37332205877. Mobile: +37379429274
www.moldmedjournal.md editor@moldmedjournal.md secretary@moldmedjournal.md