ISSN 1857-0666

Curierul medical

SCIENTIFIC MEDICAL JOURNAL

Ministry of Health of the Republic of Moldova
Nicolae Testemitsanu State University of Medicine and Pharmacy

Ministerul Sănătății
al Republicii Moldova.
Universitatea de Stat de Medicină și
Farmacie „Nicolae Testemițanu"

Министерство здравоохранения
Республики Молдова.
Государственный университет медицины и
фармации им. Н. А. Тестемицану

Issued bimonthly

Vol. 59, No 2
April, 2016

Printing House "Tipografia Sirius"
2, A. Lapusneanu str., Chisinau, MD-2004
the Republic of Moldova
www. sirius.md
Phone: (+373 22) 232352

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Index for subscription – 32130

RESEARCH STUDIES

Influence of new Schiff bases and their combinations with 3d metals on the glutathione and thiol-disulfide metabolism in the liver in physiological conditions

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Abstract

Background: Glutathione and glutathione metabolism enzymes are essential for the normal functioning of cells and tissues and maintenance of body homeostasis. The aim of our study was to investigate the influence of the new Schiff bases, and their combinations with 3d metals, on the glutathione metabolism in the liver in physiological conditions.

Material and methods: An experimental study of laboratory animals (rats) was performed. New local biologically active compounds (BAC) – CMD-4, CMD-8 and CMJ-23, were injected to healthy rats for 14 days. The content of glutathione (total, reduced and oxidized) and the activity of glutathione reductase (GR), glutathione peroxidase (GPO), glutathione-S-transferase (GST) and glutaredoxin (GRX) were determined in the liver homogenates.

Results: All tested BAC statistically significant decreased the level of reduced and total glutathione (about 20-30%), and GMJ-23 also reduced the oxidized glutathione in the liver (-48%, $p<0.05$). Influence on the activity of enzymes of glutathione metabolism was more ambiguous. Administration of CMD-4, CMD-8 and CMJ-23 did not produce conclusive changes of the enzyme activity of the glutathione metabolism, and their induction had only tendency of GR, GST and GRX activity decrease, while CMT-28 and CMT-67 preferentially modulated the activity of enzymes.

Conclusions: Local BAC exerts an individual influence on the glutathione metabolism in physiological conditions, which may be used for a particular regulation of the processes according to the cell needs.

Key words: Schiff bases, 3d metals, glutathione, liver.

Introduction

Glutathione (GSH) and glutathione metabolism enzymes are essential for normal cellular, tissue and organism functioning. Glutathione is able to neutralize the free radicals and other reactive oxygen species, interact with various xenobiotics (acetaminophen, bromobenzene, etc.), eliminate some carcinogens (formaldehyde), interfere in the metabolism of eicosanoids, adjust the level of nitric oxide (NO) through the thioredoxin system. Maintaining the optimal level of glutathione and activity of enzymes of the glutathione metabolism is essential for body homeostasis [12, 11, 13].

The reactions of production and reduction of mixed disulfides, including glutathionylation and deglutathionylation of the proteins occur permanently in the cells. These processes are catalyzed and directed by several enzymes – a complex network of thiol/disulfidoxide reductase, that are located in the endoplasmic reticulum. Glutaredoxines (GRX) and thioltransferases, which catalyze the reduction of disulfides or of the mixed disulfides of GST, belong to this group of enzymes [9, 10]. GRX and thioredoxine systems play the protective action in patients with cardiovascular diseases and cataract by deglutathionylation of cardiac and lens proteins [1, 19].

Thus, the synthesis and testing of new chemical compounds, with the potential to strengthen reserves of intracellular glutathione and control the activity of glutathione enzymes, present interest for modern biomedical science and practice.

Biologically active compounds (BAC) are represented in a very wide area of scientific research, given extremely large variety of substances both natural and synthetic, which exerts potent action on different processes in living organisms. Synthesis of new BAC and study of their prophylactic and/or therapeutic potential is permanently on the agenda of researchers, including those in Moldova.

Several laboratories and scientific groups in Moldova (Gulea A. et al. [7, 8], Macaev F. et al. [14], Gudumac V. et al. [6]) investigated BAC with various chemical structures, physico-chemical and biological actions. Previous research of Moldovan scientists has determined that these BAC have a great pharmacological potential due to their antimicrobial, antifungal, antitumor, cytostatic, immunomodulatory, bone formation induction, and hepato-protective action [16, 17, 15, 18].

The aim of our study was to establish the influence of the local BAC offered by Professor Aurelian Gulea (CMD-4, CMD-8, CMJ-23) on glutathione metabolism in the liver in physiological conditions.

Material and methods

The research was approved by the Research Ethics Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy (June 20, 2011).

Experiments were done with the male white rats weighing 160-250 g. All animals were maintained in similar standard vivarium conditions. Considering the mechanisms of regulating the circadian biological rhythms (diurnal, seasonal, etc.), and their impact on the concentration, structure and

Table 1

Influence of local BAC on the content of glutathione and protein SH-groups in the liver in physiologic conditions

Experimental groups	Total glutathione, µmol/g	Reduced glutathione, µmol/g	Oxidized glutathione, µmol/g	Protein SH-groups mol/g
Control	12,70±0,40 100%	11,24±0,49 100%	1,45±0,25 100%	22,11±1,72 (100%)
CMD-4	10,22±0,49* 80,47%	9,06±0,47* 80,60%	1,54±0,39 106,21%	19,92±0,50 (90%)
CMD-8	9,27±0,99** 73,00%	7,94±1,01* 70,64%	1,33±0,23 91,72%	22,37±0,91 (101%)
CMJ-23	9,13±0,58** 71,89%	8,36±0,57* 74,38%	0,76±0,09* 52,41%	21,75±1,50 (98%)

Note: The statistical significance of the differences compared with controls: * - p <0,05, ** - p <0,01.

distribution of intracellular components [5], experiments were done during the same season, and investigation material was collected at the same time of the day.

All studied local BAC – CMD-4, CMD-8, CMJ-23 were administered intramuscular during14 days with the daily dose 1,0 mg/kg body weight.

Animals were sacrificed under light anesthesia with sulfuric ether, and liver was taken after 24 hours from the last administration of BAC. All operations were performed at temperature +4oC. For biochemical investigations livers were subjected to homogenization. The whole process of preparing the liver homogenates was determined under specific conditions for assessing enzyme activity.

The following indices of glutathione and thiol-disulfide metabolism: the level of total, reduced and oxidized glutathione, the content of protein SH-groups, the activity of glutathione reductase (GR), glutathione peroxidase (GPO), glutathione-S-transferase (GST) and glutaredoxine (GRX) were determined in the liver homogenates according to the procedures described by Gudumac V. and co-authors [6].

All methods for determination of enzyme activity and contents of chemical compounds have been used by techniques in our modification, adapted for application to the spectrophotometer Power Wave HT (BioTek Instruments, USA) and the microplate spectro-fluorimeter Synergy H1 (Hydride Reader, BioTek Instruments, USA).

The statistical analysis of the data has been performed by non-parametrical Mann-Whitney U-test. The differences have been considered significant for p<0.05. The data are presented as a M±SEM.

Results and discussion

The research of the local Schiff base BAC showed their significant influence on the level of glutathione and content of protein SH-groups in physiological conditions (tab. 1).

It was established that content of reduced glutathione was significantly higher than of the oxidized glutathione – 11,24 ± 0,49 µmol/g tissue vs 1,45 ± 0,25 µmol/g tissue (p<0,001) in the liver of the control animals. At the same time, the amount of protein thiol groups was almost 2 times higher than of the total glutathione (p <0,001).

BAC (CMD-4, CMD-8 and CMJ-23) administration at the dose of 1,0 mg/kg body weight, induced changes of different magnitude of the contents of glutathione and protein thiol groups in the liver of the healthy animals.

The contents of total and reduced glutathione were decreased by all BAC included in this study. The level of total glutathione was decreased by CMD-4 by 20% (p<0,05 in both cases), by CMD-8 by approximately 30% (p<0,01, and respectively, p<0,05) and CMJ-23 by 26-28% (p<0,01, and respectively, p<0,05). At the same time, the level of oxidized glutathione was not statistically conclusively influenced by the local BAC. Only CMJ-23 reduced by 48% (p<0,05) the content of the oxidized glutathione in the liver of control animals (Tab. 1). Values of the protein thiol groups, as well were not changed significantly by the tested BAC. Just CMD-4 induced a descending trend of the level of protein thiol groups (-10%, p>0,05).

Local Schiff base type coordination compounds and their complexes with metals did not have significant impact on the activity of enzymes of the glutathione metabolism (tab. 2).

Table 2

Influence of local BAC on glutathione metabolism enzymes in the liver in physiologic conditions

Experimental groups	GR (nmol/s·g prot.)	GPO (nmol/s·g prot.)	GST (nmol/s·g prot.)	Glutaredoxine (nmol/s·g prot.)
Control	30,12±2,91 (100%)	29,44±2,11 (100%)	31,22±1,92 (100%)	14,16±2,37 (100%)
CMD-4	27,56±1,02 (92%)	29,44±1,73 (100%)	30,07±1,12 (96%)	13,45±3,39 (95%)
CMD-8	24,59±1,71 (82%)	30,39±2,26 (103%)	27,51±1,02 (88%)	12,73±0,89 (90%)
CMJ-23	26,92±1,96 (89%)	31,17±3,43 (106%)	28,18±1,40 (90%)	9,68±0,82 (68%)

Note: GR – glutathione reductase; GPO – glutathione peroxidase; GST – glutathione-S-transferase.

None of the studied local BAC produced significant changes of the activity of GR, GST and glutaredoxine. However, all tested BAC induced the decreasing of the GR activity by about 8-18% (p>0,05), of GST - by about 4-12% (p>0,05), and glutaredoxine - by about 5-32% (p>0,05). Glutathione peroxidase activity was not influenced by any of the studied BAC, its activity was mantained within 100-106% limits of the reference values.

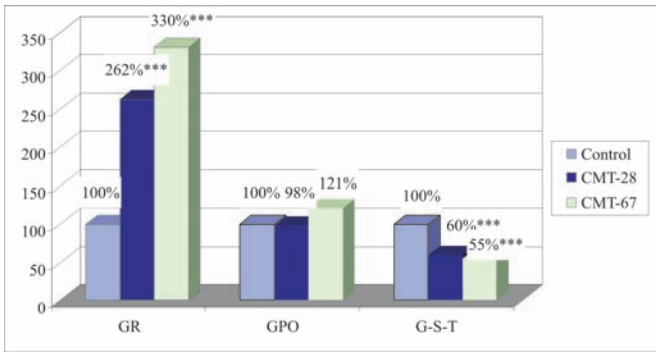

Fig. 1. Influence of CMT-28 and CMT-67 on the activity of glutathione reductase (GR), glutathione peroxidase (GPO) and glutathione-S-transferase (GST) in the liver of healthy rats (%).

Note: The statistical significance of the differences compared with controls: *** – p<0,001.

Thus, the influence of BAC – CMD-4, CMD-8 and CMJ-23 didn't change the activity of GPO, which is needed for the reduced glutathione using it for protective function against peroxides of lipids. The level of its activity was similar to the level of control animals. At that time the activity of GR, which is the enzyme for regeneration of the oxidized glutathione (GSSG) to its reduced form (GSH), decreased by action of BAC. This causes the depletion of reduced glutathione and the capacity of glutathione system to neutralize various chemical compounds.

The results of the influence of local Schiff base type with copper-containing coordination compounds - CMT-28 and CMT-67, on the activity of glutathione reductase and glutathione peroxidase in the liver in physiological conditions did not reveal statistically conclusive changes of the activity of these enzymes (fig. 1).

Both compounds increased statistically conclusive activity of GR - CMT-28 about 2.6-fold (p<0.001), and CMT-67 about 3.3-fold (p<0.001) compared to the values found in the animals of the control group. Activity of glutathione peroxidase was not influenced by CMT-28 (98%) and increased after administration of CMT-67 by 21% (p<0.01).

At the same time, CMT-67 and CMT-28 decreased statistically significant activity of GST by 40% (p<0.001) and 45% (p<0.001), compared to the values identified in the liver of the healthy animals (fig. 1).

Thus, the exertions of CMT-28 and CMT-67 provided different influence from those specific ones of CMD-4, CMD-8 and CMJ-23, which opens up possibilities for using the studied compounds in order to modulate the activity of enzymes of the glutathione metabolism. Considering that maintaining of high levels of GSH and decreased values of GSSG is cardinal for ongoing of the glutathione-dependent processes, because the glutathione realizes its majority of biological functions in reduced form, beneficial effect on the metabolism of glutathione in physiological conditions exert CMT-28 and CMT-67, which were increasing the GR activity. The enzyme converts oxidized glutathione to GSH, considerably reduces de novo synthesis of GSH and maintains the antioxidant activity of the enzymes responsible for the reduction of peroxides (GPO, GST) and disulfids (glutaredoxins) [3, 20, 21].

System GR-GSH has an important value in maintaining of the thiol-disulfide status in mammalian cells. This system plays a key role in protecting of cellular macromolecules against damage caused by free radicals that are generated in excess. Also systems GR-GSH and TrxR/TRX TrxR-TRX are involved in several cellular signaling pathways (control the activity of transcription factors and other factors that regulate apoptosis and cell division). Inhibition of mentioned pathways has dual consequences - can induce programmed cell death (apoptosis), or increase the sensitivity of cells, including tumor cell, to the action of drugs [2, 4, 10].

Conclusions

The exertions of local biologically active compounds provided the different influence on the indices of glutathione metabolism in the physiological conditions. New Schiff bases in combination with 3d-metals, CMD-4, CMD-8 and CMJ-23, caused changes in the content of total, reduced and oxidized glutathione, but did not act on the activity of enzymes of the glutathione metabolism , while CMT-28 and CMT-67 preferentially modulated the enzyme activity. Thus, for the local BAC takes place the specific individual influence on the glutathione metabolism, that may be used for a particular regulation of the processes according to the cell needs.

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Evaluation of antibiotics consumption in therapeutic intensive care department

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Abstract

Background: Monitoring of aggregate, ward-supply data and analysis of the anatomical therapeutic chemical/defined daily dose system, adjusted for bed-occupancy, provides a clear picture of antibiotics consumption frequency and time-trends within hospitals and especially intensive care departments.
Material and methods: For this study we used data of a five-year (2010-2014) period, in therapeutic intensive care department of the Emergency Medicine Institute, which show the consumption dynamics of anti-infectives for systemic use of drugs in grams and value indexes.
Results: The defined daily doses (DDD) per 1000 occupied-bed days (DDD/OBD) of antibiotics in therapeutic intensive care department decreased from 1524 in 2010 to 1206 DDD/1000 in 2014 or by 20.87%, however, it is by 11.77% higher than medium consumption of 1052.25 DDD/1000 in intensive care units with the same activity in international hospitals. The value of 54948 lei per DDD/1000 OBD in 2010 recorded a decline to 40754 lei or by 25.84% in 2014. The cost of one medium DDD from 36.05 lei in 2010 decreased to 33.77 lei or by 6.33% in 2014. The average antibiotics annual institution consumption constituting 464.1 DDD/1000 in 2014 was higher by 1.06% comparatively with medium consumption of 459.20 DDD/1000 registered in 1706 international hospitals, and by 35.31% in comparison with global consumption of 343 defined daily doses per 1000 patient-days.
Conclusions: The decrease of DDD/1000 OBD and their cost took place as a result of efforts for rational use of antibiotics during the evaluated period. Great opportunities were found for improving rational utilization of anti-infectives for systemic use.
Key words: antibiotics, defined daily dose, consumption, rational use, hospitals.

Introduction

The main function of the Therapeutic intensive care department of Emergency Medicine Institute consists in providing measures to recover patients after anesthesia of surgical, neurosurgical, traumatological and other investigations as well as from other possible critical conditions of hospitalized patients. Often these patients are exposed to multiple invasive procedures and have to administrate a multiple broad spectrum of antibiotics. At the same time, the prescriptions are often empiric and lead to overuse or misuse of antibiotics, unnecessary side effects, growth of pathogenic microbes resistance to antibiotics and increasing treatment costs. The above mentioned requires surveillance, stringent consumption control and suppose rational antibiotic prescription [1, 2, 3, 4]. Unfortunately, in the Republic of Moldova the information about antibiotics prescription, consumption patterns, and cost analysis in hospitals with only few scientific publications [5, 9, 10, 20] is rather limited.

National Scientific-Practical Centre of Emergency Medicine of the Republic of Moldova reorganized in 2014 into Emer-

gency Medical Institute (EMI), was founded in 1959. Clinical Services of EMI include: Orthopedic-Traumatology Clinic for 150 beds, Surgery Clinic for 140 beds, Neurosurgery Clinic for 80 beds, Neurology Clinic for 70 beds, Maxillo-facial clinic for 30 beds, Urology Clinic for 40 beds, Gynecology Clinic for 30 beds, Microsurgery Clinic for 30 beds, Municipal center for hemodialysis with 8 seats and 9 beds, Clinical intensive care unit for 30 beds including and Therapeutical intensive care department for 12 beds, in total the above services of the EMI include 600 beds overall. In addition to the above mentioned services include 5 emergency medical help substations and 4 out-patient Departments of traumatology and orthopedics [6].

The primary aim of the study was to evaluate institutional representative data on antibiotics utilization for five-year (2010-2014) period, in accordance with World Health Organization (WHO) requirements, directed to determine the value of Defined Daily Doses per 1000 Occupied-Bed Days (DDD/1000). That study was carried out and with the support of other two programs that were effectuated in the institution: analysis program of consumption and stocks of drugs in Anatomical Therapeutical Chemical (ATC) and pharmacotherapeutic software [7, 8]. Based on obtained data, it aimed to make conclusions on the use of anti-infectives for systemic use in the Therapeutical intensive care department and to propose recommendations for ensuring their optimization.

Material and methods

For this study we used the data of a five-year (2010-2014) period in therapeutic intensive care department of EMI for 12 beds, which show the dynamics of consumption of anti-infectives for systemic use drugs, as classified by ATC classification system of World Health Organization indicated in grams and value indexes. Statistical, analytical, mathematical, comparative, logical and descriptive were used as the methods of study.

Results and discussion

For determining the amount of DDD/1000 data about total annual consumption of antibiotics and the statistics data concerning the number of treated patients (only patients with health insurance and other free treated by the state categories of citizens), the number of bed/days (2010 = 2922; 2011 = 3327; 2012 = 3239; 2013 = 3407; 2014 = 3388) during the evaluated period in Therapeutical intensive care department were used. All in all, 36 antimicrobial remedies with different dosage of administration (both for parenteral and enteral use) for hospitalized patients' treatment in the evaluated period were used entral forms – 7 names; parenteral forms – 29 names and 3 names of both forms, which represent 23 active antimicrobial substances.

Parenteral consumption forms rate of antibiotic subgroups evaluated in DDD/1000 during 2010-2014 is shown in figure 1.

As can be observed from figure 1 in the evaluated period the average consumption annual rate of all antibiotic subgroups records a decline from 1504 in 2010 to 1082 DDD/1000 in 2014 or by 28.06%. The main consumption of 1378.16 (974.67+285.42+118.07) or 97.63% from the total in 2010 to 951.01 DDD/1000 (597.7+97.11+256.20) or 87.89% in 2014 by a decrease of 427.15 DDD or 31% during the mentioned years was registered for three subgroups: other beta-lactam antibacterials (Cefazolinum 3.0, Cefuroximum 3.0, Cefotaximum4.0, Ceftazidimum 4.0, Ceftriaxonum 2.0, Cefoperazonum 4.0), other antibacterials (Vancomycinum 2.0 and Metronidazolum 1.5) and beta-lactam antibacterials, penicillins (Ampicillinum2.0, Amoxycillinum 2.0, Amoxicillinum + Acidumclavulanicum 3.0, Ticarcillinum + Acidumclavulanicum 15.0). The mean consumption of 105.75 (35.25+65.02+5.48) or 7.03% of the total in 2010 to 125.74 DDD/1000 (59.33+51.06+15.35) or 11.62% from the total in 2014 by an increase of 19.99 DDD/1000 or 18.90% was recorded in the same period by other three subgroups: aminoglycoside antibacterials (Streptomycinum 1.0, Gentamycinum 0.2, Kanamycinum 1.0, Amikacinum 1.0), quinolone antibacterials (Gatifloxacinum 0.4, Acidumpipemidicum 0.8) and antimycotics for systemic use (Fluconazolum 0.2). The lowest consumption of 21.53 (1.37+21.53) or 1.43% of the total in 2010 to 6.61 DDD/1000 (2.07+3.54) or 0.61% from the total in 2014, with a decrease 14.92 DDD/1000 by 3.26 times was recorded in that period by other two subrgoups: amphenicols (Chloramphenicolum 3.0) and macrolides, lincosamides and streptogramins (Clarithromycinum 0.5, Azithromycinum 0.5, Lincomycinum 1.8).

In figure 2 consumption rate of enteral forms of antibacterials for systemic use in DDD/1000 during 2010-2014 is shown.

As it is seen from figure 2 the average consumption annual rate of antibiotics for oral usage increased from 19.85 in 2010 to 123.72 DDD/1000 OBD in 2014 or by 6.23 times. The highest consumption of 19.85 DDD/1000 or 100% of the

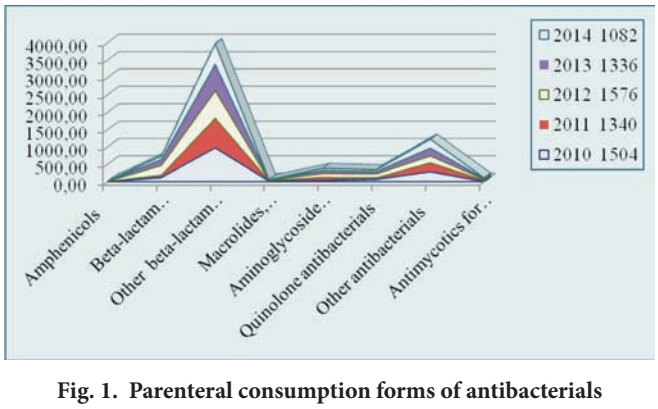

Fig. 1. Parenteral consumption forms of antibacterials for systemic use in DDD/1000.

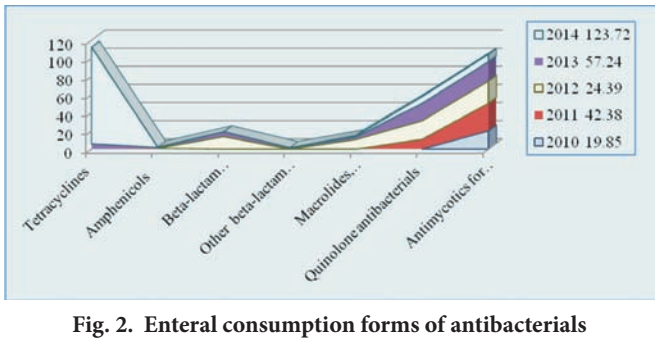

Fig. 2. Enteral consumption forms of antibacterials for systemic use in DDD/1000.

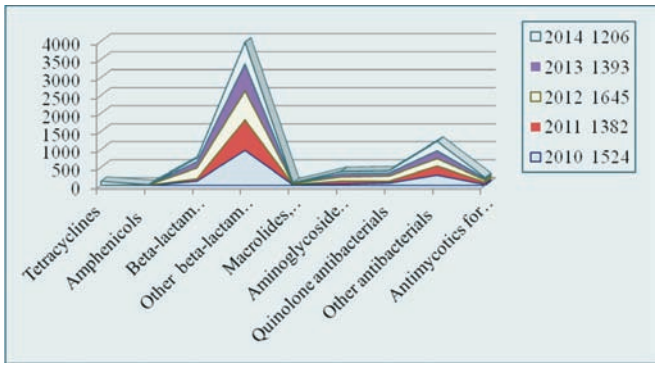

Fig. 3. The total consumption of antibacterials for systemic use in DDD/1000.

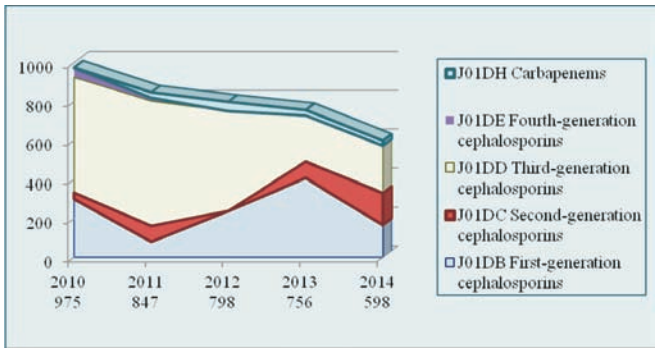

Fig. 4. Total consumption forms of other beta-lactam antibacterials for parenteral use in DDD/1000.

total in 2010 was registered for antimycotics for systemic use and 106.6 DDD/1000 or 86.16% of the total in 2014 for tetracyclines. In figure 3 the total (parenteral and enteral forms) antibiotic subgroups used rates are demonstrated.

As it can be observed from figure 3 the average aggregated annual rate for total antibiotics consumption in the evaluated period decreased from 1524 in 2010 to 1206 DDD/1000 in 2014 or by 20.87%.

The highest yearly consumption for the first 3 subgroups during mentioned years remains similar to parenteral use (other beta-lactam antibacterials, other antibacterials and beta-lactam antibacterials, penicillins) as well as for mean (aminoglycoside antibacterials, quinolone antibacterials, antimycotics for systemic use) and lower consumption (amphenicols, macrolides, lincosamides and streptogramins).

Nevertheless, though consumption of enteral forms of antibiotics registered a significant increase during the evaluated period, the total DDD/1000 consumption wasn't influenced respectively.

As stated in table 1 in the evaluated period the ratio between antibiotics DDD/1000 parenteral to enteral forms was 75.79:1 in 2010 to 8.75:1 in 2014. The percentage of parenteral forms from the total antibiotics DDD/1000 in the mentioned period decreased from 98.70% to 89.74%, as well as the enteral forms increased from 1.3 to 10.26% of the total. Similar data for the entire institution can be found in some early publications [10]. Some publications demonstrate that in terms of switch therapy, approximately 40-50% of patients admitted for intravenous antibiotics can be switched to oral antibiotics within 2-3 days [11].

From this chart one can see in the evaluated period the total consumption of parenteral forms of cephalosporin's decreased from 975 to 598 DDD/1000 or by 38.67%. Cephalosporin's first generation (Cefalexinum and Cefazolinum) represents 300.82 DDD/1000 or a share of 30.85% from the total in 2010, and 166.77 DDD/1000 or 27.88% from the total in 2014 by a decrease of 134.05 DDD/1000 or 44.57% during 5 years. The consumption of cephalosporin's second generation (Cefuroximum, Cefaclorum) recorded an increment from 32.11 DDD/1000 to 163.81 DDD/1000 or by 5.10 times during the mentioned period. Cephalosporinț's third generation (Cefotaximum, Ceftazidimum, Ceftriaxonum, Cefixim, Cefoperazonum, Cefoperazonum + Sulbactamum) demonstrates a considerable decrease of consumption from 591.72 DDD/1000 in 2010 to 241.74 DDD/1000 in 2014 or by 2.44 times. Cephalosporins G-IV and carbapemens (Meropenemum, Imipenemum+Cilastatinum) recorded a consumption lower than 50 DDD/1000.

Comparison of total consumption data of anti-infectives for systemic use evaluated in DDD/1000 in 54 intensive care units of international hospitals with the similar data of Therapeutical intensive care department of EMI is presented in figure 2.

As we can see from table 2 the average annual rate for total-hospital antibiotics utilization period in EMI decreased from 662.4 in 2010 to 464.1 DDD/1000 in 2014 or by 30%. That result was higher by 67.65 DDD/1000 or by 14.58% than the medium consumption of 396.45 DDD/1000 registered in case of 1256 international hospitals and lower by 112.66 DDD/1000 or by 36.68% in case of 450 international hospitals where the

Table 1

The ratio between DDD/1000 for parenteral to enteral antibiotic form

Years	2 010	2011	2012	2013	2014	
Parenteral	1504.44	1339.95	1576.11	1336.06	1082.36	
Enteral	19.85	42.382	68.85	57.239	123.715	
The ratio of parenteral to oral	75.79:1	31.62:1	22.89:1	23.34:1	8.75:1	
Total	1524.29	1382.33	1644.96	1393.30	1206.08	
Percentage from total	Parenteral	98.70	96.93	95.81	95.89	89.74
	Enteral	1.30	3.07	4.19	4.11	10.26

Table 2

Surveillance studies of antibiotics use in intensive care units of international hospitals, compared with the similar data in therapeutical intensive care department of EMI

Setting	Surveillance time-period	Data source Pharmacy	Data collection	Use of antibiotics in DDD/1000 bed-days over the study period
Emergency Medicine Institute	6 years (2010–2014)	dispensing records (PDR)	Annual	662.4 hospital-wide in 2010 464.1 hospital-wide in 2014
Therapeutical intensive care department of EMI	5 years (2010–2014)	(PDR)	Annual	1524.29 in 2010 1206.08 in 2014
ICU Tertiary level in Northern India [12]	In 2008	(PDR)	Annual	1086.5
Neurosurgical ICU of Germany [13]	From 2002 to 2005	(PDR)	Annual	652.0
40 ICU of Southwestern Germany non university regional general hospitals [14]	From 2001 to 2002	(PDR)	Annual	1056.0 (in medical ICU) 1169.0 (in surgical ICU) 1127.0 (in mixed ICU)
8 Tertiary intensive care unit in Hungary [15]	In 2008	(PDR)	Annual	1013.0
ICU of Military Medical Academy hospital of Bulgaria [16]	In 2011	(PDR)	Annual	1052.0
ICU of 1 university hospital in Switzerland [17x24Loeffler, JM, Garbino, J, Lew, D, Harbarth, S, and Rohner, P. Antibiotic consumption, bacterial resistance and their correlation in a Swiss university hospital and its adult intensive care units. Scand J Infect Dis. 2003; 35: 843–850 CrossRef | PubMed | Scopus (47) See all References24]	5 years (1996–2000)	(PDR)	Annual	462.0 (in surgical ICU) 683.0 (in medical ICU) 400.0 (in the entire hospital)
ICU of 1 university hospital in Greece (personal unpublished data) [18]	5 years (1998–2002)	(PDR)	Annual	982.0 (in mixed ICU)
The global antibiotics consumption [19]	From 2006 to 2008 varied little	(PDR)	Annual	343.0

mentioned medium was 634.34 DDD/1000 respectively. Other all medium consumption in 1706 international hospitals constituting 459.20 DDD/1000 was lower than consumption of 464.1 DDD/1000 in EMI in 2014 by 4.90 DDD/1000 or by 1.06% and lower by 121.1 comparatively to global antibiotics consumption of 343 defined daily doses per 1000 patient-days or by 20.09% [20].

DDD/1000 of antibiotics in Therapeutical intensive care department decreased from 1524 in 2010 to 1206 DDD/1000 in 2014 or by 20.87%, however, it is by 11.77% higher than medium consumption of 1064.01 DDD/1000 [(1x1086.5 + 1x652.0 + 15x1056.0 + 10x1169 + 10x1127 + 8x1013 + 1x1052 + 1x462 + 1x683 + 1x982):54] in intensive care units of 54 international hospitals with the similar activity.

The value cost of parenteral forms of antibacterials for systemic use per DDD/1000 in lei is shown in figure 5.

As we can see from figure 5 the average consumption annual rate per DDD/1000 in value indexes (lei) of all parenteral antibiotic subgroups recorded a decline from 54782 in 2010 to 40509 lei in 2014 or by 26.06%. The medium yearly consumption for the evaluated period with more than 5000 lei per DDD/1000 was registered for other beta-lactam antibacterials (31975.8 lei) and for beta-lactam antibacterials (10146.5 lei). Other subgroups as other antibacterials, macrolides, lincosamides and streptogramins, quinolone antibacterials, antimycotics for systemic use registered a medium yearly consumption less than 5000 lei per DDD/1000.

The value cost of enteral forms of antibacterials for systemic use per DDD/1000 in lei is presented in figure 6.

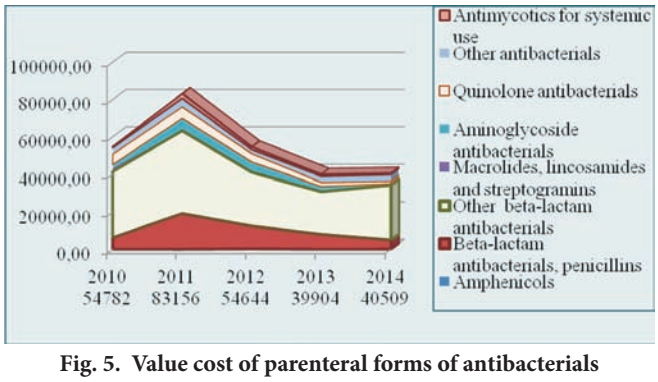

Fig. 5. Value cost of parenteral forms of antibacterials for systemic use per DDD/1000 in lei.

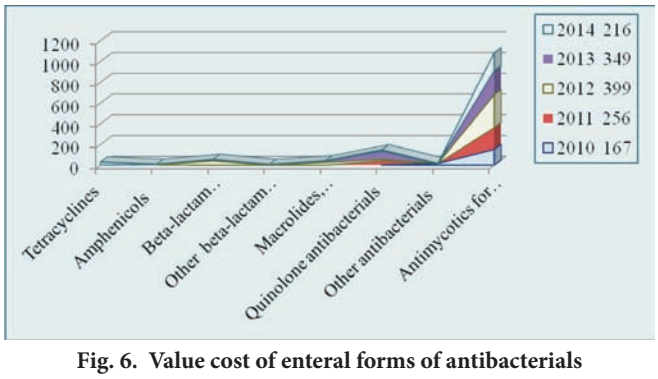

Fig. 6. Value cost of enteral forms of antibacterials for systemic use per DDD/1000 in lei.

From figure 6 it can be found that the average consumption annual rate in value indexes of all antibiotic subgroups records an increase from 167 in 2010 to 216 lei per DDD/1000 in 2014

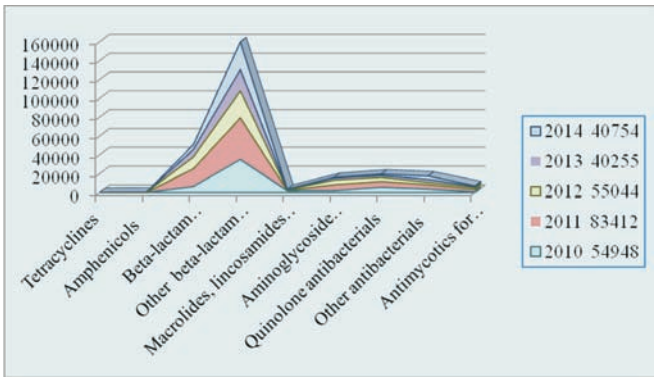

Fig. 7. Total value cost of antibacterial for systemic use per DDD/1000 in lei.

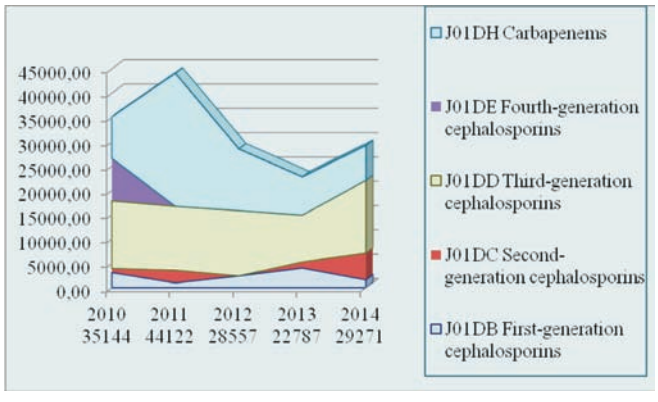

Fig. 8. Total value cost of other beta-lactam antibacterials for parenteral use of DDD/1000 in lei.

or by 29.34%. All other subgroups recorded consumption per DDD/1000 less than 100 lei. Total value cost of antibacterials for systemic use per DDD/1000 in lei is shown in figure 7.

In this chart the presented data demonstrate that the average consumption annual rate in value indexes of total antibiotics record a decline from 54948 in 2010 to 40754 lei per DDD/1000 in 2014 or by 25.84%. The highest yearly cost of DDD/1000 of the total antibacterials for systemic use represents other beta-lactam antibacterials and beta-lactam antibacterials. Introduction of coordinated cost control program and prescribed protocols established by consensus as guidelines for a rational policy in antibiotics therapy in some institutions result in decreasing of value cost during 2 years by more than 40% [21, 22].

Total value cost of other beta-lactam antibacterials for parenteral use of DDD/1000 in lei is presented in figure 8.

Figure 8 shows that the cost of DDD/1000 in value indexes (lei) of antibiotic subgroups for parenteral use recorded a decline from 35144 in 2010 to 29271 lei in 2014 or by 16.72%. As an exception from the decline was 2011 year with the higher consumption of 44122 lei per DDD/1000. An increment in cost per DDD/1000 was recorded by the second generation of cephalosporin's from 859.24 in 2010 to 5525.00 lei in 2014 or by 6.43 times. Third cephalosporin's generation slightly increased cost per DDD/1000 from 13884.1 in 2010 to 14948 lei in 2014 or by 7.66%. A decrement in the evaluated period was recorded by the first generation of cephalosporins from 3113.05 lei per DDD/1000 to 1605.5 or by 48.43% and carbapenems from 8508.83 lei per DDD/1000 to 7192.70 or by 18.29%.

To determine the cost of one medium DDD of antibacterials for systemic use separately for parenteral and enteral forms, the cost sum of DDD/1000 per DDD/1000 was divided respectively. The cost of one medium DDD of antibiotics in lei for parenteral and enteral forms and total is shown in table 3.

As we can see from table 3 in the evaluated period the cost of one medium DDD recorded a slow increase from 36.41 lei in 2010 to 37.43 lei in 2014 or by 2.80% for parenteral forms, a decrease from 8.41 to 1.75 lei or by 4.80 times for enteral forms and from 36.05 to 33.77 lei or by 6.33% for one total DDD. In chronological way for the evaluated years the ratio between the cost of one medium DDD of parenteral to enteral forms was respectively 4.32:1; 9.78:1; 5.97:1; 4.81:1 and 21.38:1.

Treatment patterns, antibiotics stewardship activity, potential opportunities for early switch from intravenous to oral formulations [23] and measures necessary for preventing and strengthening antimicrobials resistance and nosocomial infections will lead to potential cost savings per every eligible patient [24, 25, 26].

Table 3

Cost of 1(one) DDD of antibiotics in lei for parenteral and enteral forms and total

Year/ cost (in lei)	2010	2011	2012	2013	2014
Parenteral cost in lei per DDD/1000	54781.50	83156.00	54644.40	39904.00	40509.00
Enteral cost in lei per DDD/1000	166.88	255.99	399.13	355.18	216.23
Total cost in lei per DDD/1000	54948.38	83411.99	55043.53	40259.18	40725.23
Parenteral DDD/1000	1504.45	1339.95	1576.11	1336.06	1082.36
Enteral DDD/1000	19.85	42.382	68.85	57.239	123.715
Total DDD/1000	1524.29	1383.83	1644.95	1394.77	1206.07
Cost in lei per 1(one) DDD	36.05	60.43	33.61	28.89	33.77
Parenteral cost in lei per 1(one) DDD	36.41	62.06	34.67	29.87	37.43
Enteral cost in lei per 1(one) DDD	8.41	6.04	5.80	6.2	1.75

Conclusions

1. The DDD/1000 of antibiotics in therapeutical intensive care department decreased from 1524 in 2010 to 1206 DDD/1000 in 2014 or by 20.87%, however, it was by 11.77% higher than medium consumption of 1052.25 DDD/1000 in intensive care units of 54 international hospitals. The consumption of parenteral forms constituting 1504.44 or 98.70% from the total in 2010 and 1082.36 DDD/1000 or 89.74% from the total in 2014 had an overall decrease of 29.06%. For enteral forms the stated data were 19.85 or 1.30% from the total in 2010 to 123.72 DDD/1000 or 10.25% from the total in 2014 or an increase by 6.23 times respectively.

2. The value of 54948 lei per DDD/1000 OBD in 2010 recorded a decline to 40754 lei in 2014 or by 25.84%. The cost of one medium DDD from 36.05 lei in 2010 decreased to 33.77 lei in 2014 or by 6.33%. International experience demonstrates that introduction of coordinated cost control program and prescribed protocols can lead to a decrease of anti-microbials treatment cost during 2 years by more than 40%.

3. 36 antimicrobial remedies with different dosage of administration with: enteral forms – 7 names, parenteral forms – 29 names and with both forms – 3 names, that represent 23 active antimicrobial substances in the period from 2010-2014 were evaluated.

4. Besides the drugs consumption evaluation in DDD that permits to improve rational use of medical remedies in hospitals of the Republic of Moldova, daily practices in antimicrobial treatment of potential opportunities for early switch from intravenous to oral formulations, coordinated cost control program and prescribed protocols established as guidelines for a rational policy in antibiotics therapy, and the last but not the least the measures for preventing and strengthening antimicrobials resistance and nosocomial infections as well will lead to growing treatment quality of hospitalized patients and potentially saving institutional budgets.

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Curierul medical, April 2016, Vol. 59, No 2

Photometric determination of Fe (III) with sulfosalicylic acid using the standard addition method in oral drops Ferropol

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Abstract

Background: Iron deficiency in the human body decreases the hemoglobin level and so iron deficiency anemia occurs. In this case, doctors recommend the use of medical preparations in different forms: solid or liquid.
Material and methods: In order to determine the quantity of Iron (III) in different medical preparations that contain Iron (III)-hydroxide polymaltose complex the analysis starts with the breakdown of the complex in acidic media. At the interaction of the mineral acids with Fe (III) hydroxide polymaltose complex during heating the brown color disappears and Fe (III) ions pass into the solution.
Results: It was shown that iron forms with sulfosalicylic acid a red-violet compound in acid media that absorbs light at a wavelength of 505 nanometers. The object of the study consists in the photometric method of the analysis of Fe in liquid forms, with the addition of sulfosalicylic acid using the standard addition method. Due to the fact that in a basic medium Fe (II) gently oxidizes to the Fe (III), then by the photometric method with sulfosalicylic acid it is quantitatively determined even Fe (III), as well as the summary content of Fe (II) and Fe (III) in the analyzed solution.
Conclusions: A new method of iron analysis was developed. It can be recommended to determine the iron in liquid medical preparations.
Key words: iron deficiency, photometric determination of iron, analysis of iron, quality assurance.

Introduction

Iron is an important element of the human body. The deficiency of iron in the body decreases the hemoglobin level in the blood and causes iron-deficiency anemia. To combat this phenomenon doctors recommend the usage of medicinal preparations as active substance containing Fe (II) or Fe (III) in various forms: solid [1] or liquid [2]. In this case, the development of methods for the quality analysis of dosage forms of Fe remains quite actual.

Ferropol oral drops contain as active substance 50 mg/ml Fe (III) hydroxide with polymaltose, which is equivalent to 50 mg / ml of iron. The macromolecular compound of the Fe (III) is stable, it does not eliminate free iron ion form, by the structure it is similar to natural compounds of Fe. Due to this similarity, Fe (III) passes from the intestine into the blood only by the way of active absorption, which explains the impossibility of poisoning the preparation unlike simple salts of Fe, which absorption depends on concentration.

Material and methods

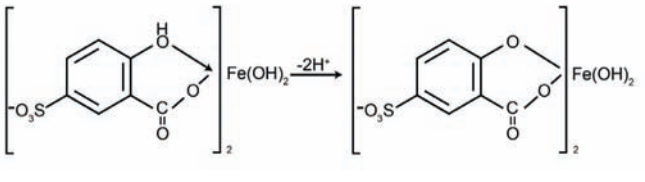
For the quantitative determination of Fe (III) in various liquid formulations (forms) which contain the complex compound of the Fe (III) hydroxide with polymaltose and have a dark brown color, firstly it is necessary to decompose this complex macromolecular compound in the acid medium [2]. At the interaction of the mineral acids with Fe (III) hydroxide polymaltose complex during heating the brown color disappears and Fe (III) ions pass into the solution.

Both, the absorption spectrum of the standard solution of Fe (III), as well as the spectrum of the test solution to Fe (III), obtained from the oral drops of Ferropol, with sulfosalicylic acid in an acid medium have been recorded using the spectrophotometer Agilent 5483. The absorption of test solutions was measured using photoelectric colorimeter KFK-2MP (КФК-2МП) at a wavelength of 490 nm, using the cells with absorption thickness layer of 1 cm. The pHs of the solutions were measured using laboratory ionomer I-160M (И-160М), using as an indicator electrode –the glass electrode.

Results and discussion

The sulfosalicylic acid is an organic ligand, which is used for the photometric determination of iron in different stages of oxidation [3]. In the acid medium (pH 1.8 - 2.5) the solution forms a Fe (III) complex in ratio to metal: ligand is equal to 1: 1 red - violet absorbing electromagnetic radiation maximum at λ = 505 nm [4, 5]. This complex compound is used in practice for the photometric determination of Fe (III) in the presence of Fe (II).

In a basic medium (9 <pH <11.5) Fe (III) with sulfosalicylic acid forms a complex compound of yellow color, which maximally absorbs electromagnetic radiation at λ = 424 nm. Ratio metal: ligand complex is 1: 2 and actually it is supposed that in the basic medium occurs only the complex deprotonation [6]:



Due to the fact that in a basic medium Fe (II) gently oxidizes to the Fe (III), then by the photometric method with sulfosalicylic acid it is quantitatively determined even Fe (III), as well as the summary content of Fe (II) and Fe (III) in the analyzed solution [5, 7].

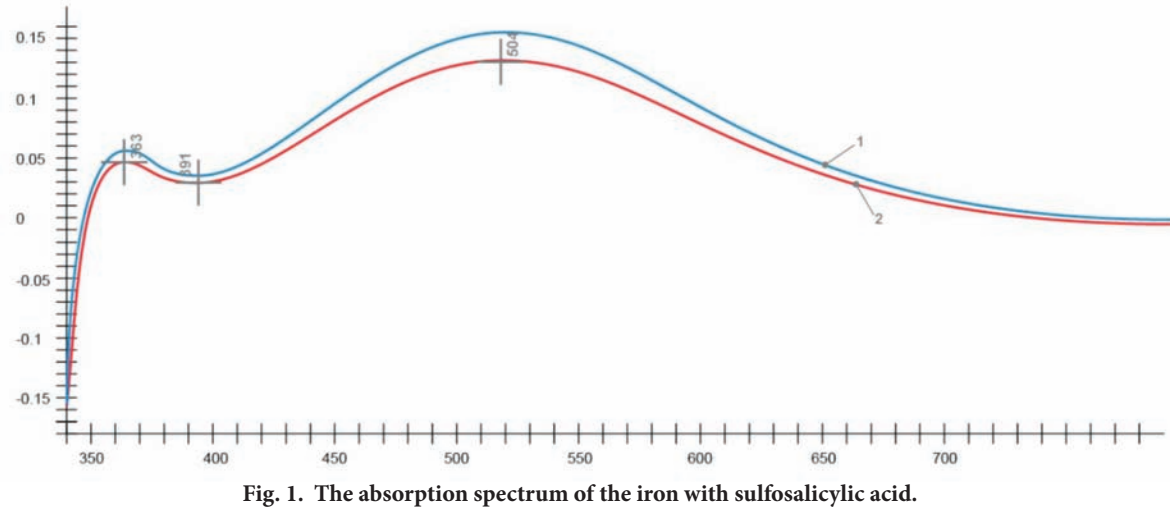


Fig. 1. The absorption spectrum of the iron with sulfosalicylic acid.

In this work, we have studied the possibility of photometric determination of Fe (III) in the oral drops Ferropol by standard addition method.

Preparation of standard solution of Fe (III). In this study is used the double salt $(NH_4)2SO_4 \cdot 6H_2O \cdot FeSO_4$ – commonly named as „Mohr salt", which was recrystallized from distilled water. A sample of this salt with a weight of 0.17553g was passed quantitatively into a volumetric flask with the capacity of 500 ml, it was dissolved in distilled water, acidified by 0.5 ml of concentrated HNO_3 and the solution was heated on the electric hob until boiling. After cooling the solution was acidified by 3 ml of 1M H_2SO_4, diluted with distilled water till mark and was homogenized. The molar concentration of the standard solution was equal to $4.4762 \cdot 10^{-4}$ mol / l and the content of Fe (III) in it was equal to 0.05 mg / ml.

The solution with sulfosalicylic acid of 10% was prepared from the reagent $C_6H_6O_6S \cdot 2H_2O$ and it was filtered after the preparation.

Preparation of sample for analysis. Firstly, was established the exact mass of vial with cap. Then with automatic dosing pipette brand DACpette were measured and passed 200 µl of Ferropol oral drops into vial and it was exactly weighed. Then the content of the vial has been quantitatively moved into a volumetric flask with the capacity of 200 ml. Then were added 5 ml of 1M H_2SO_4 . It was placed in a water bath at 100°C, where hydrolyzation of Fe (III) polymaltose complex took place and 2 minutes after the brown color of the solution completely disappeared. After cooling the solution was diluted till mark with distilled water and homogenized.

In some flasks with the capacity of 50 ml was added the same volume of the initial test solution of Ferropol oral drops (2.0 ml). Starting from the second flask in each flask were added various volumes of the standard solution of Fe (III) as shown in table 1. Then in all flasks were added 5 ml of sulfosalicylic acid, the solutions were diluted up to the mark with distilled water, then homogenized and left to stand for 10 minutes.

In parallel in a volumetric flask with the capacity of 50 ml there were added 5 ml of sulfosalicylic acid and the volume was made up to the mark with distilled water (Solution for comparison).

The preventive experiments showed that the absorption spectrum of the solution to be analyzed of Fe (III), obtained from the oral drops of Ferropol, with sulfosalicylic acid is similar to the absorption spectrum of a standard solution of Fe (III) with this ligand in the acid medium and maximally absorbs the electromagnetic radiation in the visible region of the electromagnetic spectrum at a wavelength of 504 nm (fig. 1).

The spectrophotometric standard addition method is a modification of the method of the comparison and in practice it is used to determine the unknown concentration or the mass of the substance by the calculation method or graphics.

By the method of calculating the two absorbances are compared between the two of them: the first A_x – absorbance of a solution which only contains the analyte with the unknown mass mx, and the second A_{x+a} – absorbance of the solution which contains the same known mass mx of the substance to be analyzed, and further addition of the standard solution with ma mass.

If these two solutions are prepared in two flasks of equal capacity, then comparing the absorbances easily results the relation to calculate the unknown mass m x Fe (III) in the solution to be analyzed of Ferropol oral drops, to which are measured the absorbances A_x and A_{x+a}

$$m_x = \frac{A_x}{A_{x+a} - A_x} \cdot m_a \quad (1)$$

The addition mass (m_a, mg) in the solution where the absorbance A_{x+a} was measured was calculated according to the relation:

$$m_a = m_i \cdot V_i, \quad (2)$$

where:

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

V_i – the volume of the initial standard solution of Fe (III) which was added, ml.

The mass of Fe (III) (m_{Fe}, mg) in the initial solution of oral drops Ferropol was calculated by the equation:

$$m_{Fe} = m_x \cdot \frac{V_0}{V_1}, \quad (3)$$

in which:

V_0 – the capacity of the flask with initial solution of Ferropol oral drops, ml;

V_1 – fraction of the initial solution of Ferropol oral drops, which has been taken for the preparation of the two solutions, with the absorbances of A_x and A_{x+a}, ml.

By combining the equations (1), (2) and (3), we obtain the relation to calculate the mass of Fe (III) (m_{Fe}, Mg) in the initial solution of oral drops of Ferropol:

$$m_{Fe} = \frac{A_x \cdot m_i \cdot V_i \cdot V_0}{(A_{x+a} - A_x) \cdot V_1}, \quad (4)$$

all the notes see above.

In the pharmaceutical liquid forms content of the active substance is usually expressed in mg/ml. In its turn the volume (V) of the liquid of the pharmaceutical form is connected to the mass and its density by the equation:

$$V = \frac{m_p}{\rho} \quad (5)$$

where:

m_p – The mass of the liquid pharmaceutical sample, g;

ρ – density of liquid form, g/ml.

Introducing the equation (5) in the relation (4) we obtain the final formula to calculate the mass of Fe (III) in the oral drops of Ferropol (m Fe, mg/ml):

$$m_{Fe} = \frac{A_x \cdot m_i \cdot V_i \cdot V_0 \cdot \rho}{(A_{x+a} - A_x) \cdot V_1 \cdot m_p}, \quad (6)$$

all the notes see above.

Table 1

Data for the determination of Fe (III) with sulfosalicylic acid in the acid media of the oral drops Ferropol by the photometric method of standard addition

No	V1, ml	Vi, ml	ma, mg	pH	Ax și Ax+a	mFe , mg/ml
1	2,0	-	-	2,07	0,067	-
2	2,0	1,0	0,050	2,04	0,101	49,26
3	2,0	1,5	0,075	2,03	0,118	49,26
4	2,0	2,0	0,100	2,01	0,134	50,00
5	2,0	2,5	0,125	2,00	0,149	51,07
6	2,0	3,0	0,150	1,99	0,167	50,25

(V_0= 200 ml; m_i=0,05 mg/ml; m_p=0,2272 g; ρ=1,136 g/ml)

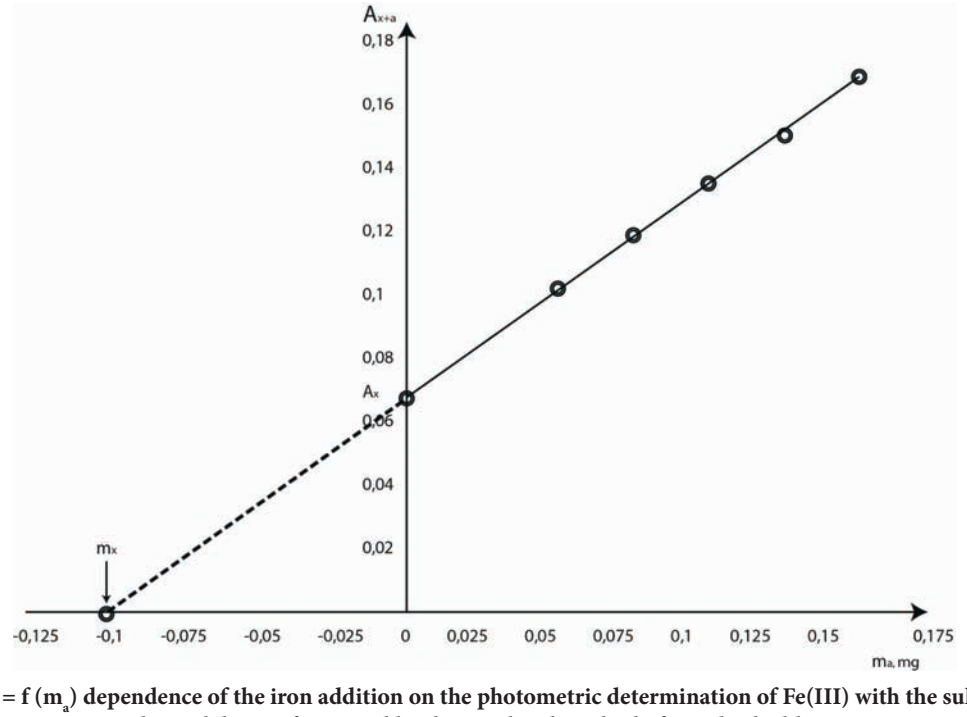


Fig. 2. The $A_{x+a} = f(m_a)$ dependence of the iron addition on the photometric determination of Fe(III) with the sulfosalicylic acid in the oral drops of Ferropol by the graphical method of standard addition.

The obtained experimental data and the results of calculating the mass of Fe (III) in the oral drops of Ferropol by the relation (6), as well as the value of pH in the studied solutions are presented in table 1.

The data in table 1 were statistically processed to obtain the average content of Fe (III) Ferropol oral drops equal to 49.97 ± 0.94 mg/ml, having a 0.95% confidence interval.

In the graphical method of standard addition the solution absorbance $A_{x+a} = f(m_a)$ is a straight line that intersects on the ordinate axis the absorbance value Ax (see Fig. 2). At the extrapolation of this line to the intersection with the x-axis was obtained the mean value of Fe (III) mass in the solutions where the absorbances A_{x+a}: - $m_a = m_x$ were measured. In the Fig. 2 we find that mx = 0.10mg Fe (III).

The content of Fe (III) (m_{Fe}, mg/ml) in the oral drops of Ferropol, using the data from Table I has been calculated according to the relation:

$$m_{Fe} = \frac{\overline{m}_x \cdot V_0 \cdot \rho}{V_1 \cdot m_p}, \quad (7)$$

(all of the notes see above) and we obtained m_{Fe} = 50 0 mg/ml.

Conclusions

It was elaborated a new method of photometric analysis of iron in the oral drops of Ferropol using the sulfosalicylic acid by the method of standard addition. The elaborated method can be recommended to analyze the iron in the liquid pharmaceutical forms.

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Curierul medical, April 2016, Vol. 59, No 2

Diagnostic algorithm of cranial deformities in children with severe neurological disorders

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Abstract

Background: Cranial deformities (plagiocephaly) generate various health problems in children, fact that may influence neurophysiological development both craniometrically and functionally. The aim of this study was to develop an algorithm for diagnosis of cranial deformities and dental alveolar appearance in children with delayed neurologic sequelae.

Material and methods: 370 children with neurological disorders were examined by a team (neurologist, orthodontist, oral and maxillofacial surgeon, plastic surgeon). It was recorded in the individual questionnaire: a) the degree of cranial deformity; b) the form of dentoalveolar anomaly c) type of brain dysfunction, which allowed the elucidation of correlations, previously unknown, depending on the nature and intensity of specific clinical and evolutionary manifestation.

Results: Cranial deformities in children were highlighted in 50% of cases. Relation boys:girls was 1:1. Positional cranial deformities in 25% of cases are associated with a different degree (the first degree – 1%, the second degree – 7.3%, the third degree – 50%, the fourth degree – 4.1%, the fifth degree – 1%), and 1.96% of craniostenosis and 21% of other deformities in children with severe cognitive and motor disorders. Dental and maxillar deformities in the sagittal plane were found in 28% of children with and in 25% without cranial deformities. Dental crowdings were found in 54% of children with and in 30% without cranial deformities. While the protrusion of the mandible and dental spaces were found in 8% and 21% in children with and 10% and 18% in children without cranial deformities.

Conclusions: In conclusion we found that cranial deformities in children with neurological disorders are present in 50% of cases. In the diagnosis of dental and maxillar deformities, it is necessary to determine when cranial deformities can lead to installation of malocclusions in children. In order to confirm the results of this study, it is necessary to make studies on anatomic disorders of cranial basis, cranial vault.

Key words: cranial deformities, children, neurological disorders.

Introduction

The United States Public Health Service has launched the campaign "Back to Sleep" to support the supine position for newborns during sleep. As a result of this campaign, the United States Craniofacial Anomalies Center has marked an increase in incidents of craniofacial skeletal deformities in infants [5, 8]. If in 1974 the presence of cranial and facial deformities was reported as 1newborn of 300 newborn children, so in 1996 the incidence increased to 1of 60 living newborn children [2, 5, 10]. Increased incidence of craniofacial deformities generated many aesthetic and functional problems in these children. Collett B. R. (2013), Hutchison B. L. (2012), Miller R. I. (2000) [3, 4, 5] found cognitive disorders, that were more highlighted in subsequent periods of neuropsychological development. 39.7% of children with plagiocephalies, examined during the preschool period, required special care, additional exercises, and individual curriculum. Collet B. R. (2013), Miller R. I. (2000) [3, 6] studying the scores of cognitive development of children with cranial and facial deformities, found a high risk of reduction of IQ coefficient during the school period, compared with healthy children.

The facts that the maxillar is directly joined to 11 and indirectly to the rest of cranial bones, and that skull anatomical changes could influence the development of the dentoalveolar system, are less reflected in the specialty literature [11, 13].

In literature we found no data available on the evolution of cranial deformities, forms of cranial deformities, about frequency of cranial deformities and their correlation with the presence of malocclusions in children of school age and children from various health groups. Thus, several authors found that orthodontic treatment is needed in children of school age from the group of healthy children [2, 14]. In this context it is known that the frequency of malocclusions simultaneously increases [1, 7, 9] in children with neurological disorders. Thus, in studies made on 124 and 381 children with neurological disorders [7] in South Africa, 58% to 74% of cases needed orthodontic treatment. On the other hand, scientific studies reflect only the correlation of behaviour disorders that could influence changes of dentoalveolar system. In this study we mapped out to examine the incidence of cranial deformities and their correlation with the presence of dentomaxillar deformities in school-age children with neurological disorders.

Material and methods

370 children with neurological disorders were examined in 3 residential institutions with special education. The children in the study had various forms of neurological diseases: mental retardation [F70 - F79] – 43 [11.6%] children; cerebral palsy and other paralytic syndromes [G80 - G83] – 45 [12.1%] children; episodic paroxysmal disorders [G40 - G47] – 52 [14.0%] children; sequelae of inflammatory diseases of the central nervous system [G09] – 65 [17.5%] patients; neurotic, stress and somatoform disorders [F40 - F48] – 69 [18.6%] children; congenital malformations, chromosomal deformities and anomalies [Q00 - Q99] – 36 [9.7%] children; other disorders of the nervous system [G90 - G99] – 60 [16.2%] children.

Examination of malocclusions in children was carried out after the simplest method, which determines the presence or absence of malocclusion. Also, certain information was specified, considering the difficulty of examinations of these children [12]. The simplest were examinations in the anterior segment, determining malocclusion in the sagittal plane, the presence of dental crowding and dental spaces. Data were recorded in questionnaires reflecting: 1 – occlusion (Angle classification); 2 – overjet; 3 – overbite; 4 – correlation dental alveolar in the anterior segment. Angle classification was used to determine interdental relations in the anterio-posterior position. This classification divides occlusions into first class, second class (two subdivisions) and third class, using first molars as a reference point. In the absence of molars canines were used. Examination standards were used for diagnosis of malocclusions. Normal occlusion: the term includes minimal deviations from ideal parts that do not generate aesthetic and functional changes.

Spaces. Segment considered „spaces" is determined when there is no proximal contact between the teeth.

Crowding – the segment that contains overlapping teeth, or lack of spaces in the dental arch for tooth eruption.

Skull shape of all children was examined by manual palpation methods and, in case of detection of cranial deformities, a bandage on deformed skull was applied to confirm the presence of deformities and to determine their form. Children with cranial deformities were divided into three categories: 1 – craniostenosis; 2 – plagiocephalies; 3 – other cranial deformities (microcephalies, hydrocephalies etc.).

The results were analyzed using "Epi-info-2002" and „Excel" from „Microsoft Office" package. The data were interpreted as M ± m (average error) by means of the t-Student test. All statistical methods were obtained from the "Statistics for Windows" version 6. The difference was regarded as conclusive when $p < 0.05$.

Thus, according to the criteria reflected in tabular list of diseases ICD-10-AM (10th Revision of the International Statistical Classification of Diseases and Related Health Problems) 7 groups of children with neurodental health problems were obtained.

Results

In this study the distribution of children by gender was equal [b:g = 1:1]. Table 1 elucidates the frequency of cranial deformities in relation to clinical manifestations of neurological pathology. Thus, in 1.9% of cases (7 children) were found deformities of the skull, specific to craniostenosis with an increase (0.8% of cases) in group of children with cerebral palsy [CP] and other paralytic syndromes. In other 44 children (11.9%) cranial deformities, identical to positional plagiocephalies, were found, especially in children with predominantly neurological disorders and in 10 children [2.7%], mainly with motor disorders, cranial deformities were not included in classifications of plagiocephalies and craniostenosis.

Thus, the distribution of children by cranial deformities, shape and degree of clinical manifestation of neurological pathology at first sight is not statistically authentic (P>0,05). Also, depending on the structure of neurological pathology it is found impressive that cranial deformities, on the one hand, in 48.9% of cases were associated in the late period more frequently with: a) cerebral palsy (CP) and other paralytic syndromes (15 4% of cases); b) congenital malformations (9.5% of cases); c) episodic and paroxysmal disorders (8.4% of cases); d) mental retardation (7.3%) and less frequently with: a) other diseases of the central nervous system (CNS) [4.3% cases]; b) neurotic, stress and somatoform disorders (2.1%) and c) the consequences of neuroinfections (1.9% cases).

On the other hand, cranial deformities in the late period, in children examined more frequently, prevailed in: a) episodic and paroxysmal disorders [68.9%]; b) CP and other paralytic syndromes (65.5%); c) other CNS disorders (48.5%); d) the consequences of neuroinfections (43.6%); e) congenital malformations (41.7%); f) mental retardation (35.1%) and g) neurotic, stress and somatoform disorders (28.6%) (table 1).

Table 1

Number of children with positional cranial deformities and their distribution by neurological disorders

Pathology	Group I without cranial deformities		Group II with cranial deformities		Forms of cranial deformity						Total	
					Craniostenosis		Plagiocephaly		Other cranial deformities			
	n	%	n	%	n	%	n	%	n	%	n	%
G80 - G83	30	8,1	57	15,4	3	0,8	44	11,9	10	2,7	87	23,5
Q00 - Q99	49	13,3	35	9,5	1	0,27	24	6,4	10	2,7	84	22,7
G40 - G47	14	3,8	31	8,4	2	0,6	19	5,1	10	2,7	45	12,2
F70 - F79	50	13,5	27	7,3	0	0.00	7	1,9	20	5,4	77	20,8
F40 - F48	20	5,4	8	2,1	1	0,27	6	1,6	1	0,27	28	7,6
G90 - G99	17	4,6	16	4,3	0	0.00	9	2,5	7	1,9	33	8,9
G09	9	2,4	7	1,9	0	0.00	4	1,1	3	0,8	16	4,3
Total	189	51,1	181	48,9	7	1,9	113	30,4	61	16,5	370	100

X^2=27,829; P>0,05

Table 2

Maxilla protrusion in relation to cranial deformities

Maxilla protrusion	Forms of cranial deformity										
	Without cranial deformity		With cranial deformity							TOTAL	
			Craniostenosis		Plagiocephaly		Other cranial deformity		Total		
	n	%	n	%	n	%	n	%	n	n	%
Present	46	25	3	37,5	30	31	18	23	51	97	26
Absent	139	75	5	62,5	68	69	61	77	134	273	73
Total	185	100	8	100	98	100	79	100	185	370	100

X^2=2,161; P>0,05

Table 3

Protrusion of the mandible in relation to the cranial deformities

Mandible protrusion	Forms of cranial deformity										
	Without cranial deformity		With other cranial deformity							TOTAL	
			Craniostenosis		Plagiocephaly		Other cranial deformity		Total		
	n	%	n	%	n	%	n	%	n	n	%
Absent	166	90	6	75	92	94	72	91	170	366	91
Present	19	10	2	25	6	6	7	9	15	34	9
Total	185	100	8	100	98	100	79	100	185	370	100

X^2=3,770; P>0,05

In order to emphasize clinical neurodental features, the results of examinations in the oral cavity in relation with or without cranial deformities and delayed neurologic sequelae are revealed. In tables two and three the presence of dental and maxilla deformities in the sagittal plane was identified. Thus, in 370 children with neurological disorders, 185 [50%] children did not have cranial deformities. So, in this group of children cranial deformities were found with a high frequency – 50%.

In this study, together with the detection of frequency of cranial deformities in children with severe neurological disorders, we have analyzed the clinical dentomaxillary condition of the anterior segment of the maxilla and mandible and their correlation with cranial deformities. Table 2 shows reflected data on the anterior displacement in the sagittal plane of the maxilla (maxilla protrusion). From 370 children maxilla protrusion was detected in 97 (26%). In the group of children without cranial deformities from 185 children in 46 [25%] cases maxilla protrusion was found and in 139 (75%) children was not found. At the same time, from 185 children with cranial deformities in 51 (28%) children the anterior displacement of the maxilla in the sagittal plane was found. We want to mention, that in the group of children with cranial deformities of craniostenosis and plagiocephaly type the anterior maxillar displacement in the sagittal plane considerably surpasses (31% to 38%). In the group of children with other cranial deformities, this pathology was found on a smaller percentage of children (23%) (table 2).

The relations of the maxilla to the mandible and cranial deformities were presented in table 3. From 370 children the anterior displacement of the mandible in the sagittal plane (mandible protrusion) was observed in a small number of children in comparison with the maxilla - 34 children (9%). At the same time, from 185 examined children with disorders the mandible protrusion was found in 10 (10%). In those with cranial deformities, from 185 children 15 (8.1%) were children with anterior displacement of the mandible in the sagittal plane, especially 6 children (3.2% cases) presented plagiocephalies, 2 (1.0%) craniostenosis and 7 (3.7%) cases of other deformities with anterior displacement of the mandible were found (table 3).

159 children (43% cases) in the study group were found with Angle class I, 60 children (16.2%) – Angle class II and 23 (9.2%) – Angle class III. We have to mention that in group with Angle class II 20 children (5.4%) with cranial deformities of plagiocephaly type, 3 cases (0.8%) of craniostenosis and 7 cases (1.9%) with other cranial deformities were found. A smaller number of children with Angle class III were found: 23 cases (6.2%), of which 5 children (1.4%) with plagiocephaly, 2 (0.5%) with craniostenosis and 2 (0.5%) with other deformities.

Outcomes of study, aimed to determine the dental bone relation in the anterior segment of maxilla and mandible of the examined children, are shown in Tables 4 and 5. The tables show that dental crowdings were observed in 123 cases (33%) of all examined children: 66 (54%) children from that group

Table 4

Distribution of children with cranial deformities and dental-bone relation (anterior segment)

Dental crowding	Forms of cranial deformity										
	Without cranial deformity		With other cranial deformity							TOTAL	
			Craniostenosis		Plagiocephaly		Other cranial deformity		Total		
	n	%	n	%	n	%	n	%	n	n	%
Absent	128	69	7	87,5	62	63	50	63	119	247	67
Present in maxilla	15	8	0	0	9	9	9	11	18	33	9
Present in mandible	20	11	1	12,5	14	14	11	14	25	46	12
Present in both	22	12	0	0	13	13	9	11	23	44	12
Total	185	100	8	100	98	100	79	100	185	370	100

X^2=4,327; P>0,05

were with cranial deformities and 57 children (30%) without cranial deformities. We noticed that dental crowdings are present more frequently in children with plagiocephaly than with other cranial deformities 36 (55%).

The presence of dental spaces in the anterior segment in children with cranial deformities has been detected less frequently in children with neurological disabilities compared with the presence of dental spaces – 74 (20%) cases. In the group of children with cranial deformities dental spaces were found in 18% of cases and in 21% of cases in children without cranial deformities. In Tables 4 and 5 we find that dental crowdings in children with cranial deformities are encountered more frequently in relation to dental spaces.

Discussion

The presence of cranial deformities has been addressed in literature on the specialty for more than 20 years, with the launch of the campaign "back to sleep", after a considerable increase in the number of children with cranial deformities was noticed [2, 8, 5]. In countries from the former Soviet Union they are less elucidated [15]. Together with this campaign many publications on the side effect of these pathologies in the development of preschool children have appeared.

Considering the high risk of harm to these children during their neuropsychiatric development, a great attention from parents, teachers, physicians [3, 4, 6, 10] is payed to this problem. In this study we tried to make a correlation between children with neurological disorders, cranial deformities and dentomaxillary status. We found the presence of cranial deformities in 50 % of children with neurological disorders. 30.4% of cases were positional plagiocephalies, 2% were craniostenosis and 16.5% were other cranial deformities, which more or less may affect neuropsychological development of a child.

Thus, every second child with severe neurological disorders has cranial deformities in a ratio of 1:2. In specialized literature data on the role of cranial deformities in the structure of neurological entities were not ilucidated. In this study it was observed that some neurological pathologies – CP and other paralytic syndromes (15.4% of cases); b) birth defects [9.5% of cases]; c) and episodic and paroxysmal disorders (8.4% of cases); d) mental retardation (7.3%) – present a higher incidence of cranial deformities.

The frequency of occlusal pathologies of neurological disorders among children has been studied by many authors [9, 1, 7]. Some authors have concluded that dentomaxillar deformities are more common in children with neurological pathologies compared to healthy children [1, 9]. They reasoned

Table 5

Distribution of children with cranial deformities and dental-bone relation (anterior segment)

Dental spaces	Forms of cranial deformity										
	Without cranial deformity		With cranial deformity							TOTAL	
			Craniostenosis		Plagiocephaly		Other cranial deformity		Total		
	n	%	n	%	n	%	n	%	n	n	%
Absent	145	78	8	100	83	85	60	76	151	296	80
Present in maxilla	16	9	0	0	6	6	13	16	19	35	10
Present in mandible	11	6	0	0	2	2	3	4	5	16	4
Prezent in both	13	7	0	0	7	7	3	4	10	23	6
Total	185	100	8	100	98	100	79	100	185	370	100

X^2=11,295; P>0,05

the high frequency of malocclusions with a type of disability, with the frequency of adjacent pathologies and behavioral vices. Onyeaso C.O., (2002) [7] comparing the frequency of malocclusions in children with neurological disorders and healthy children found no statistically authentic difference of occlusion disorders. At the same time, Birgit Theander and coauthors (2001) [12], Fabio Ciuffoli and coauthors in 2005 [14] doing a literature review, found that the incidence of abnormalities of occlusion in healthy children of school age ranges from 39% to 93%. Also these authors in their studies have found that only 11.9% of children examined had ideal occlusion criteria. The results of different authors vary, probably, because of the geographical location of the country where the study was made, its socio-economic condition, and examination methods used in each case [12,14]. In this study we found that in children with neurological disabilities Angle Class I was present in 21.4% and Angle Class II in 9.5% of cases. This study results showed a frequency of Angle index similar to Onyeaso C.O. and coauthors and lower than at other authors – Utomi I. L. 2009 [9]. It is to note in this context that data can be unclear because of examination difficulties of children with various forms of behavior. Also, in this study we found that the high number of children with deformities of jaws of Angle type I and II is more common in children with cranial deformities (21% of children diagnosed with plagiocephalies and 35% of children with craniostenosis). Changes in sagittal occlusion (protrusion of the maxilla and mandible) were made by several authors trying to make a priority in all dentoalveolar anomalies [7, 9, 12, 13, 14]. Onyeaso C.O. and coauthors [7] in their conclusions found a similar relation in the group of children with disorders and also found no differences in percentage in the group of healthy children and those with disorders. In this study we found out that the protrusion of the maxilla prevails (29%) compared with the mandible (9%). Also, protrusion of the maxilla was higher among children with cranial deformities (28%) compared to children without cranial deformities (25%). They were found most frequent in children with craniostenosis.

We also found big differences in results of authors on the presence of dental crowding and spaces in children. Some authors found the prevalence of dental crowding [14,12] while others [9,7] of dental spaces. Onyeaso C. O. (2002) [7] is the author who considers there is no percentage differences of these abnormalities in the group of healthy children and those with neurological disorders. We found that of 370 children examined, 33% had dental crowdings and 20% - dental spaces. In 185 children with deformities 36% were found with dental crowdings and 18% with dental spaces, compared with those who did not have cranial deformities 31% and 22%.

Conclusions

In conclusion we found that cranial deformities in children with neurological disorders are present in 50% of cases. A prevalence of abnormalities of occlusion was found in children with cranial deformities. When determining malocclusions, their prophylaxis and treatment, it is necessary to take into account the changes that occur in the cranial vault and the base. In order to confirm these findings, it is necessary to clarify when cranial deformities may influence the development of the dentomaxillary system.

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The predictive factors for positive molecular-genetic assay in patients with pulmonary tuberculosis from Chisinau city

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Abstract

Background: One of the most important tuberculosis control action is the early detection, especially of multidrug-resistant tuberculosis. Cultural methods remain the golden standard for pulmonary tuberculosis diagnosis. The microscopic identification of acid-fast-bacilli in sputum is still a worldwide used method for TB detection. The low sensibility of conventional microscopic methods endangers the actual epidemiological situation. Starting with 2012, the genetic-molecular technology – Xpert MTB/RIF Assay - was implemented in the Republic of Moldova. The assessment of initial experience of Xpert MTB/RIF Assay is compulsory for improving the early case detection. The aim of the study is the assessment of extrinsic factors predictable in positive Xpert MTB/RIF patients.

Material and methods: A retrospective, selective, descriptive and case-control study was performed. There were enrolled 361 new pulmonary tuberculosis patients, diagnosed and hospitalized in the Muncipal Clinical Hospital of Phthysiopneumology of Chisinau in the period of 01.01.2014 - 01.01.2015. The patients were disributed into 2 groups: I - 174 patients with positive Xpert MTB/RIF Assay result; II - 187 patients with negative Xpert MTB/RIF Assay result. Investigations were performed according to the National Clinical Protocol – 123 Tuberculosis in adults.

Results: The male gender was predominating above the female gender in both groups: 67,8% vs 32,2% in the 1st group and 55,6% vs 44,4% in the 2nd group. According to the economical status, disadvantaged patients were predominating in the 1st group (71,3%), as compared with the 2nd group (50.8%), with the degree of concludence, p<0,001. The hystory of household TB contact was predominating in the 1st group – 17,2% vs 9,1% in the 2nd group, (p<0.05).

Conclusions: The implementation of Xpert MTB/RIF Assay improves the early TB detection and the prompt initiation of an adequate treatment regimen, according to the susceptibility testing results.

Key words: tuberculosis, Xpert MBT/RIF Assay, risk factors.

Introduction

Tuberculosis (TB) is one of the most important challenges for the health care system of any state. World Health Organization (WHO) declared tuberculosis a global emergency in 1993 [6]. In 2014, 9 million new cases were registered globally, the Republic of Moldova ranking among 30 countries with the biggest burden of multidrug-resistant tuberculosis (MDR-TB). One of the most relevant tuberculosis control action represents the early case detection, especially of MDR-TB cases [21]. So, precocious treatment of early detected new case is considered the most efficient tool for interrupting the epidemiological chain of infectious transmission [9]. The cultural methods still remain the golden standard for pulmonary tuberculosis diagnosis. Conventional microscopy for identification of acid-fast-bacilli is the first step in the TB detection algorithm and the largely extended investigation method for the TB diagnosis [18]. The low sensibility of conventional microscopy methods endangers the actual epidemiological situation. There is not enough sensibility in some individual cases, and the long duration in obtaining of culture results make difficult to achieve Millennium Goals [13, 17]. In addition, WHO established conditional recommendations to use Xpert MTB/RIF assay in adults, children and persons with HIV presumed to have TB (not especially MDR-TB), or for testing the non-respiratory specimens targeting the diagnosis of extrapulmonary TB [14, 20].

Xpert MTB/RIF assay represents an in-vitro diagnostic medical device, owned by Cepheid Company. Xpert MTB/RIF assay used with Cepheid Xpert MTB/RIF system is a semi-nested, quantitative, real-time polymerase chain reaction test used for the DNA detection of all Mycobacterium tuberculosis complex species and rifampicine resistance mutations of the rpoB gene [7]. Xpert MTB/RIF system integrates Xpert device, a computer and a barcode reader. The system automates sample processing, nucleic amplification and detection of the target sequences of rpoB gene. Primers used by the system amplify the portion of the rpoB gene, containing 81 base bars. So, the technique is able to differentiate the wild-type sequence and mutation in the core region of the rpoB gene. The system requires the use of a single-use disposable Xpert cartridge that contains all the reagents necessary for developing of polymerase chain reaction process. The cartridge also contains the sample processing control that ensures the adequate processing, and the Probe Check Control (PCC) that verifies reagent rehydration, tube filling in the cartridge, probe integrity and dye stability.

Any biological specimen (sputum, bronchoalveolar washout, cerebrospinal fluid, etc.) can be processed considering that it requires the minimum of 2 ml of sample volume. For the highest sensibility, the collection procedure, the storing and transportation should be performed at maximum 35°C, less than in 3 days, and from 2 to 8°C, for 4 – 10 days after the specimen harvesting [5, 8]. The Xpert System generates results measured according to fluorescent signals. Several standard results must be known for the appropriate interpretation of Xpert MTB/RIF system: 1. MTB detected & RIF resistant means that MTB target is present and mutation of rpoB gene is detected; 2. MTB detected & RIF susceptible means that MTB target is present and no mutation of rpoB gene has been detected; 3. MTB not detected – MTB target is not detected within the sample. Despite of clearly defined interpretations,

the test results must be always correlated with the laboratory and clinical data of the investigated patient [11].

The approved data established that three sputum samples per patient examined through Xpert MTB/RIF assay show the sensitivity among culture positive specimens on average of 97.3%, and among smear positive patients – on average of 99.5%. The specificity compared with non-tuberculosis patients was 97.9%. In the same three specimens the sensitivity for RIF resistance (rpoB gene mutation) detection among phenotypic (culture) RIF resistant patients was identified in amount of 96.1%, and specificity in phenotyping RIF sensitive patients was 98%. Error rates vary from 3% to 4%. The sensitivity is slightly decreased in a single sputum sample. Assessing the threshold of analytical sensitivity, it was argued that the biological sample must contain at least 131 colony forming units (CFU) per ml of sample with the confidence interval ranging from 106.2 CFU to 176.4 CFU. Experimentally, 9 strains of non-tuberculosis mycobacteria were mixed in sputum samples at a concentration of 106 CFU/ml, in all cases being obtained negative results at Xpert MTB/RIF Assay [2]. However, the negative result does not exclude active tuberculosis that leads to the necessary application of other important diagnostics approaches in the suspected TB patients [15]. This test couldn't be used for the treatment outcomes evaluation (success or failure), because the MTB DNA persists for a long time after the antimicrobial therapy [7].

In actual epidemiological conditions the Republic of Moldova encounters a sharp increase of MDR-TB incidence [9]. In 2011, the rates of drug-resistance were 26% among new cases and 64% among previously treated cases [12]. Starting from April 2012, the Xpert MTB/RIF technology was implemented in 15 districts and municipality laboratories, as well as in penal institutions. In total there were procured 25 Xpert MTB/RIF systems. A large number of laboratory staff was trained to use the new system. The initial experience of Xpert MTB/RIF Assay is important to be assessed for the confirmation of its role in shortening the time for TB diagnosis (including resistant forms) [11], especially in a high burden region as Chisinau municipality, the Republic of Moldova.

The aim of the study is the comparative research of the predictors in patients with Xpert MTB/RIF positive and Xpert MTB/RIF negative results.

Objectives: 1) Comparative assessment of the risk factors of pulmonary tuberculosis, according to the results of Xpert MTB/RIF assay. 2) Assessment of the predictive factors in the study groups.

Material and methods

It was performed a retrospective, selective, descriptive and case-control study, targeting the features of pulmonary tuberculosis in patients, diagnosed and hospitalized in the Municipal Clinical Hospital of Phthysiopneumology of Chisinau in the period of 01.01.2014-01.01.2015. Including criteria of the 1st Group: age > 18 years old; patient with pulmonary tuberculosis established as a new case; positive Xpert MTB/RIF Assay; including criteria of the 2nd Group: age > 18 years old; patient with pulmonary tuberculosis established as a new case; negative Xpert MTB/RIF Assay. The total number of 361 cases was distributed into 2 groups: the 1st group (1st Group) included 174 patients and the second group (2nd Group) included 187 patients. The collection of primary material involved the extraction of data from medical record forms. The individual schedule included information about: anamnesis, clinical examination, results of radiological investigations (chest radiography, plane tomography, and computer tomography), results of microbiological investigations (smear microscopy by Ziehl-Neelson coloration and culture on classic solid medium Lowenstein-Jensen or liquid medium). Investigations were performed according to the National Clinical Protocol – 123 Tuberculosis in adults [12]. Statistical analysis methods used in the study were: comparative, synthesis, discriminate analysis. The mathematical and statistical assessment was carried out by checking the quantitative and qualitative features. The accumulated material was tabled in simple and complex groups. Statistical study was performed using Microsoft Excel XP soft. The predictability value of each involved factor was calculated using the two by two tables. Relative risk and confidence interval were calculated according to the established formula [14]. The interval of 1.2 to 1.6 was assessed as a low predictive factor, 1.6 to 2.4 – as a mild predictive factor, and more than 2.5 – as a high predictive factor [14].

Results and discussion

Among 174 patients with positive Xpert MTB/RIF Assay from the 1st Group, 103 (59.2±3.73%) microscopic positive Ziehl-Neelson staining cases were determined by conventional sputum smear microscopy, also were identified 142 (81.6 ± 2.94) culture positive cases at solid and liquid media. Rifampicine resistance was established in 63 (36.2±3.64%) cases of the 1st Group.

In the 2d Group were included 187 Xpert MTB/RIF negative patients, 9 (4.8±1.56%) of them were positive acid-fast smears and 28 (14.9 ± 2.60%) were culture positive at solid (Lowenstein-Jensen) and liquid media (BACTEC MGIT). So, Xpert MTB/RIF Assay can't replace conventional microbiological methods.

Considering the analysis based on multivariate logistic regression, the burden of microscopic acid fast bacilli positive patients is high, being appreciated by Relative Risk (RR), RR=3.22 (95% CI: 2.63 - 3.96).

General characteristics, social, economical, and health insurance-related determinants of patients with pulmonary tuberculosis

The sex distribution of patients established the predominance of male sex versus female sex in both groups: 118 (67.8 ± 3.54%) males in comparison with 56 (32.2 ± 3.54%) females in the 1st Group, with the degree of significance $p<0.001$, as well as in the 2nd Group - 104 (55.6 ± 3.63%) men in comparison with 83 (44.4 ± 3.63%) women, without achieving the concludence. Male-to-female sex ratio was 2.11/1 in the 1st Group and 1,25/1 in the 2nd Group. By comparing the groups, it was

identified a significant difference between gender distribution among the investigated groups. The male sex showed a low predictability in the patients with positive Xpert MTB/RIF Assay, assessed by RR=1.32 (95% CI: 1.04 - 1.67). The data are presented in the table 1.

The distribution of patients into age groups identified a similar proporion of patients in both groups. The largest representative group was the age group of 31-40 years old: 51 (29.3±3.45%) patients from the1st Group versus 48 (25.7 ± 3.19%) patients from the 2nd Group, followed by the 21-30 age group: 44 (25.3 ± 3.29%) patients of the 1st Group and 47 (25.1 ± 3.172%) cases of the 2nd Group. The distribution in other age groups was similar (table 1). Regrouping the above exposed data in two subgroups (18 - 40 years and more than 41 years) did not identify any difference between the prevalence of patients of less than 40 years comparing with older patients in the same group, as well as when comparing both groups (table 2). Considering that male sex and young age represented the significant features for both groups, it was identified that male gender was the most significant factor versus the young age in both groups. So, the young age was a neutral predictor in patients with positive Xpert MTB/RIF test assessed by RR=1.08 (95% CI: 0.8 - 1.34).

Economic characteristics of patients with pulmonary tuberculosis

The distribution of patients according to the economic status showed that the number of patients with an economically-steady state (employed) was identified in less than 1/3 part of the 1st Group and in 1/2 part of the 2nd Group. So, the employees were more frequently identified in the 2nd Group versus the 1st Group: 92 (49.2 ± 3.65%) and 50 (28.7 ± 3.43%), respectively (p<0.001).

Table 1

Distribution of patients according to sex and age groups

Sex	1st Group (n=174)		2nd Group (n=187)		P
	N	M± m(%)	N	M ± m (%)	
Men	118	67.8 ± 3.54	104	55.6 ± 3.63	<0.01
Women	56	32.2 ± 3.54	83	44.4 ± 3.63	<0.01
<20 years	9	5.1 ± 1.67	10	5.3 ± 1.64	>0,05
21 - 30 years	44	25.3 ± 3.29	47	25.1 ± 3.172	>0,05
31 - 40 years	51	29.3 ± 3.45	48	25.7 ± 3.19	>0,05
41 - 50 years	35	20.1 ± 3.03	27	14.4 ± 2.57	>0,05
51 - 60 years	25	14.4 ± 2.65	25	13.4 ± 2.48	>0,05
> 60 years	10	5.7 ± 1.76	28	14.9 ± 2.60	>0,05

Table 2

Distribution of patients according to the age determinants

Age	1st Group (n=174)		2nd Group (n=187)		P
	N	M± m (%)	N	M ± m (%)	
Less 40 years	104	59.8 ± 3.71	105	56.2 ± 3.62	>0.05
More 41 years	70	40.2 ± 3.71	82	43.9 ± 3.62	>0.05

Table 3

Distribution of patients according economic status

Economical Status	1st Group (n=174)		2nd Group (n=187)		P
	n	M ± m (%)	N	M ± m (%)	
Employed	50	28.7 ± 3.43	92	49.2 ± 3.65	<0.001
Unemployed	83	47.7 ± 3.78	61	32.6 ± 3.42	<0.01
Retired	6	3.4 ± 1.38	12	6.4 ± 1.79	>0.05
Students	10	5.7 ± 1.76	4	2.1 ± 1.05	>0.05
Disease disability	9	5.2±1.67	10	5.3 ± 1.64	>0.05
Labor migrant	15	8.6 ± 2.12	8	4.3 ± 1.48	>0.05
Special situation	1	0.6 ± 0.57	0	0	>0.05

Table 4

Distribution of patients according to economical status

Economical Status	1st Group (n=174)		2nd Group (n=187)		P
	n	M± m (%)	n	M ± m (%)	
Steady-state	50	28.7 ± 3.43	92	49.2 ± 3.65	<0.001
Disadvantaged	124	71.3 ± 3.43	95	50.8 ± 3.65	<0.001

Table 5

Distribution of patients according to the insurance status

Insurance Status	1st Group (n=174)		2nd Group (n=187)		P
	n	M ± m (%)	n	M ± m (%)	
Insured	76	43.7 ± 3.76	127	67.9 ± 3.41	<0.001
Uninsured	98	56.3 ± 3.76	60	32.1 ± 3.41	<0.001

Table 6

Distribution of patients according to the civic status

Civic Status	1st Group (n=174)		2nd Group (n=187)		P
	n	M ± m (%)	n	M ± m (%)	
Married	60	34.5 ± 3.60	86	45.9 ± 3.64	<0.05
State marriage	8	4.6 ± 1.58	1	0.5 ± 0.53	>0.05
Unmarried	59	33.9 ± 3.58	79	42.2 ± 3.61	>0.05
Divorced	32	18.4 ± 2.93	17	9.1 ± 2.10	<0.01
Widow	15	8.6 ± 2.12	4	2.1 ± 1.05	>0.05

Table 7

Distribution of patients according to the civic state

Civic Status	1st Group (n=174)		2nd Group (n=187)		P
	n	M ± m (%)	n	M ± m (%)	
In couple	68	39.1 ± 3.69	87	46.5 ± 3.64	>0.05
Single-civil	106	60.9 ± 3.69	100	53.5 ± 3.64	>0.05

Unemployed represented the most expressed group, and were more often identified in the 1st Group versus the 2nd Group: 83 (47.7 ± 3.78%) and 61 (32.6 ± 3.42%), respectively (p<0.01). Retired patients, disease disabled and students were revealed at the same level in both groups. Labor migrants were an important part of patients from both groups (table 3).

So, economically disabled patients that included all non-economically productive patients, such as unemployed, retired and students were most prevalent in the 1st Group - 124 (71.3 ± 3.43%) patients comparing with 95 (50.8 ± 3.65%) cases in the 2nd Group. Economical vulnerability was identified as middle predictive factor in patients with positive Xpert MTB/RIF test assessed by RR=1.66 (95% CI: 1.29 - 1.2.14) (table 4).

Health insurance related issues

Health insurance represents the major condition for accessing the health care in the Republic of Moldova. General statistics demonstrated that the uninsured part of Moldovan citizens ranges from 10% to 25% of total population, depending on the demographical state (more frequent in the rural area), ethnic origin (ethnic minorities are more frequently uninsured), and other socially disadvantaged conditions. In 2014, 971.331 uninsured persons were identified in the Republic of Moldova [24]. In spite of the free of charge tuberculosis care, the lack of medical insurance determines a delayed detection and the lack of social assistance, the deficiency of active screening, the difficulty in the follow-up evaluation, all of these leading to lower treatment outcomes. In the Republic of Moldova, there are several categories of population that benefit of free insurance coverage: children till 18 years old, students of higher educational institutions, pregnant women, disabled persons with high and medium degree of disablement, retired persons, unemployed registered at the local territorial agencies, persons who take care of severely ill

Table 8

Distribution of patients according the harmful habits

Harmful habits	1st Group (n=174)		2nd Group (n=187)		P
	n	M± m (%)	N	M ± m (%)	
Tobacco smoking	138	79.3 ± 3.07	152	81.28 ± 2.82	>0.05
Drug use	3	1.7 ± 0.99	2	1.1 ± 0.75	>0.05
Alcohol abuse	64	36.8 ± 3.66	27	14.4 ± 2.57	<0.05

persons, mothers with 4 and more children, socially disadvantaged families benefiting of social assistance [25]. So, including all above mentioned individuals, the uninsured patients were more frequently identified in the 1st Group comparing to the 2nd Group: 98 (56.3 ± 3.76%) versus 60 (32.1 ± 3.41%) patients, respectively, p<0.001 (table 5). Uninsured state was identified as middle predictive factor in patients with positive Xpert MTB/RIF Assay and was assessed by RR=1.68 (95% CI: 1.33 - 2.14).

When studying the civil status, it was identified a higher rate of married patients in the 1st Group and a similar rate of married and unmarried patients in the 2nd Group. By comparing the groups, it was identified the prevalence of married patients in the 2nd Group versus the 1st Group: 86 (45.9 ± 3.64%) versus 60 (34.5 ± 3.60%), respectively, with a low degree of significance (p<0.05). On the other hand, the divorced patients predominated in the 1st Group comparing with the 2nd Group: 32 (18.4 ± 2.93%) versus 17 (9.1 ± 2.10%) cases, respectively, with high degree of significance (p<0.01) (table 6).

By redistributing patients into 2 groups, according to the civil status, the prevalence of married, recognized as in couple individuals was identified in the 2nd Group [87 (46.5 ± 3.64%)], comparing with cases in the 1st Group [68 (39.1 ± 3.69%) cases] and the single-civil state patients in the 2nd Group [100 (53.5 ± 3.64%) cases] compared with the 1st Group [106 (60.9 ± 3.69%) cases] without achieving the degree of significance (table 7). So, single civil state was identified as neutral predictive factor in the patients with positive Xpert MTB/RIF test being assessed by RR=1.17 (95% CI: 0.93 - 1.46).

Considering all above exposed data, a rank diagram with the most relevant general, economical and social characteristics of the pulmonary tuberculosis patients, according to the Xpert MTB/RIF test result was made. According to the schematic representation, the most relevant social features of patients with pulmonary tuberculosis are: disadvantaged economical state of the patients, followed by the male sex, single civil status, young age of the patients, lack of obligatory/ compulsory health insurance policy (fig. 1).

Associated harmful habits at patients with pulmonary tuberculosis

During the study were identified such harmful habits with the biggest impact on all stages of the pathogenesis of tuberculosis as: tobacco smoking, alcohol abuse, and illicit drug use. The most prevalent addiction in both groups was tobacco smoking that affected 1/3 of all investigated patients: 138 (79.3 ± 3.07%) cases in the 1st Group versus 152 (81.28 ± 2.82%) cases in the 2nd Group. The subgroup of alcohol abusers was less prevalent: 64 (36.8±3.656%) cases in the 1st Group versus 27 (14.4 ± 2.570%) cases in the 2nd Group, p<0.05. A few drug users were detected in both groups (table 8).

Public health related issues with impact on tuberculosis epidemiology

Migration has the major impact on the spread of different strains of tuberculosis. In Western Europe, the most of MDR-TB cases are diagnosed in immigrants from Eastern Europe. Short-term travelers and short-term residents are the most prevalent part of the immigrational population and the most vulnerable group. Most of them have an illegal state in the hosting country, that determins the lack of health and social insurance. Consequently, they couldn't access the screening methods and perform an effective anti-TB treatment. So,

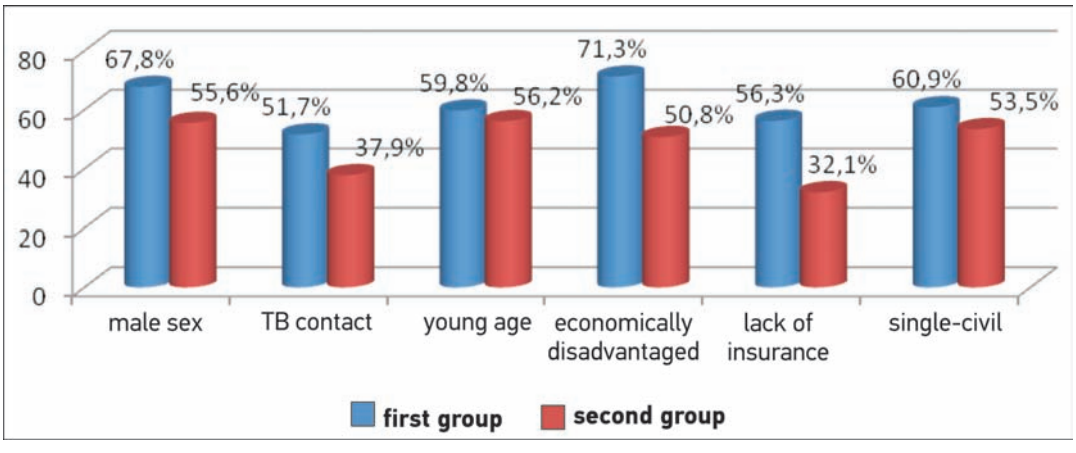

Fig. 1. Hierarchy of biologically-related and social determinants of pulmonary tuberculosis.

Table 9

Distribution of patients according to the characteristics of epidemiological cluster

Index	1st Group (n=174)		2nd Group n=187		P
	n	M ± m(%)	N	M ± m (%)	
Household/ Family contact	30	17,2 ± 2.86	17	9.1 ± 2.1	<0.05
Close contact	25	14.37 ± 2.65	34	18.18 ± 2.82	>0.05
Institutional/ Penitentiary contact	10	5.7 ± 1.76	13	6.9 ± 1.86	>0.05
Total	65	37,4 ± 3,66	64	34.2 ± 3.47	>0.05

Table 10

Relative risk from multivariate logistic regression model assessing factors associated to Xpert MTB/RIF Assay (n=361)

Factors	Relative Risk	95% CI	P
Male sex	1.32	1.04-1.67	<0.01
Young age (Less than 40)	1.08	0.8-1.34	>0.05
Economically disadvantaged state	1.66	1.29-2.14	<0.001
Uninsured state	1.68	1.33-2.14	<0.001
Single-civil state	1.17	0.93-1.46	>0.05
Epidemiological danger by being microscopic positive case-index	3.22	2.63-3.96	<0.001
Household TB Contact	1.467	1,145-1.88	<0.05

external labor migrants were 15 (8.6±2.12%) cases in the 1st Group and 8 (24.24 ± 7.46%) individuals - in the 2nd Group.

Detention, as a public health issue, exposes a high threat for multidrug resistant mycobacterial infection and active tuberculosis development. Patients, who started the treatment during the detention period and then are released from the detention/prison, have an increased risk to be lost from follow up, due to the lack of interventions to ensure the continuity of tuberculosis treatment. There were limited cases of ex-detainees in both groups: 10 (5.75 ± 1.76%) cases in the 1st Group and 13 (6.95 ± 1.86%) cases - in the 2nd Group.

Epidemiological risk factors of patients with pulmonary tuberculosis

For the TB control and interruption of epidemiological chain, it is very important to early identify the index-case. The most epidemiological danger represents MDR-TB household contact and the contact with a deceased. So, in our study were identified 3 groups of patients according to index case. The first group of TB clusters included the household or family contact, a part of this group was represented by the MDR-TB contact and the contact with a deceased. In the next group were included the patients with close contact, followed by the patients from penitentiary institutions.

Household contact prevailed in the 1st Group, 30 (17,2 ± 2,86%) patients compared to 17 (9,1 ± 2,1%) patients from the 2nd Group, with the degree of significance, p <0.05, and close contact (labor, neighborhood) was identified in 25 (14.97 ± 2.97%) cases in the 1st Group compared to 34 (18.18 ± 2.82%) cases in the 2nd Group, without achieving the degree of significance. Less prevalent was identified the epidemiological impact received by the institutionalization. Ex-prisoners were 10 (5.7 ± 1.76%) patients in the 1st Group and 13 (6.9 ± 1.86%) cases in the 2nd Group, without the degree of significance (table 9).

MDR-TB index-cases contact was identified in 4 (2.3 ± 1.13%) cases in the 1st Group and in 2 (1.1 ± 0.75%) cases in the 2nd Group, without the degree of significance; 3) dead patient contact was identified in 13 (7.5 ± 1.99%) cases in the 1st Group and in 5 (2.7 ± 1.18%) cases in the 2nd Group, without the degree of significance.

So, household TB contact was identified as a low predictive factor in the patients with positive Xpert MTB/RIF test, being assessed by RR=1.467 (95% CI: 1.145 - 1.88).

Impact of extrinsic determinants in patients with the positive Xpert MTB/RIF Assay results

By assessing all above exposed data that express the general, social, economic, and insurance-related features of patients with pulmonary tuberculosis, positive/negative Xpert MTB/RIF test through multivariate logistic regression statistical model, it was identified the prevalence only of several extrinsic factors: male sex, economically disadvantaged state, TB contact, lack of insurance (table 10).

Actual study revealed that epidemiological danger of patients with positive Xpert MTB/RIF was reduced by shortening the duration of tuberculosis diagnosis.

Conclusions

This study was first realized in Municipal Clinical Hospital of Phthysiopneumology of Chisinau.

Despite of multiple extrinsic factors assessed in the study, the multivariate logistic regression model determined that male individuals, young age, single state and household TB contact had low risk to be positive at Xpert MTB/RIF Assay, assessed by Relative Risk; economically disadvantaged patients and patients with lack of insurance had middle risk and microscopic positive patients had high risk to be positive at Xpert MTB/RIF assay.

The use of Xpert MTB/RIF could improve TB control program and epidemiological situation by earliest TB detection for achieving the epidemiological indicators, recommended by WHO.

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Considerations on structural organization of the aorta

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Abstract

Background: This article deals with a number of structural features of the human aorta which arouse great interest for clinicians recently. The following items were highlighted: a) the dependence of the aortic morphology upon the constitutional body types; b) the impact of aortic structural features on its affections; c) functional anatomy of the fat pads; d) glomic structures of the aorta; e) location of the reflexogenic areas of the aorta; f) some specific features of aortic syntopy; i) urgent need to improve terminology.

Material and methods: The study was performed on subjects coming to autopsy not more than 24 hours after death. Their mean age was from 16-week fetuses up to 96 years. A number of research methods have been used: morphometry, histological examination, immunohistochemical method, coloring with Schiff reagent, injectional investigation and anatomical preparation. A total of 354 human aortas were examined: 109 of them were selected for morphometry; 40 - for histological examination, 40 – for coloring with Schiff reagent, 20 – for injectional investigation; 69 mediastinal complexes were subjected to anatomical preparation. The presence of the lymph vessels in the wall of the aorta was confirmed by means of immunohistochemical method.

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

Conclusions: Relationship between aortic morphology and body types was revealed. The variability of the shape, size, location and contents of the fat body of ascending aorta was described. Macroscopic and microscopic structural specific features of the aorta were studied from the point of application.
Key words: aorta, fat body, vasa vasorum, glomus, reflexogenic zone, lymph collector.

Introduction

There is hardly to name another blood vessel in human body whose morphology has been so largely reported in scientific papers, as aorta. It seems as if everything is already known: its ontogenesis, morphogenesis, macro- and microstructures, properties of its walls, sources of nerve supply and blood supply. The major anatomic structures of the heart and aorta have been well described, documented and named. Two aspects attract attention here:

- Multitude of articles published by clinicians, especially cardiosurgeons, from many countries who treat heart and aortic diseases, deal with problems of the lack of morphological evidence which lead to postoperative complications and the ways to prevent them.
- At the same time, articles on the morphology of the aorta have been published rarely for the last two decades.

Having studied more than 800 scientific papers on the morphology and physiology of the aorta, it is surprising, that today, there are many uncertainties in this field of study.

Today, a frequent use of the word "enigma" in description of the heart and aorta by clinicians and anatomists means insufficient studies of these central links of the circulatory system. Multitude of articles published by clinicians, especially cardiosurgeons, from many countries who treat the diseases of the heart and aorta deal with the problem of the lack of morphological evidence that lead to a number of postoperative complications and the ways to prevent them.

Aorta is an essential component in biomechanics of the circulatory system, which has increasingly drawn the attention of the physicians. The available data, regarding the morphological organization of this magistral blood vessel are no longer sufficient to solve practical problems, since cardiovascular diseases have become a leading cause of death; the ageing of the population accounts for the increased risk of these diseases, respectively. Certain structural elements of the aorta, which until recently showed no interest, are of great clinical importance, at the moment, as well as their study and have become particularly actual, nowadays. The shape and parameters of various portions of the aorta, as well as, the relations between them should be considered in surgical planning and performing of this vessel plasty. Their studies are necessary for understanding the pathogenesis of the aortic diseases.

Early achievements of 1967 in cardiovascular field, including morphological aspects, made the first heart transplant possible. At present, the advanced cardiovascular surgery requires new studies in morphology. Despite the unprecedented advances in diagnosis and treatment of cardio-aortic diseases, cardiovascular diseases have become the leading cause of death in both industrialized and developing countries. This fact has been confirmed by statistics on all the continents. Thus, the cardiovascular diseases mortality rate, in 2010 made up 39.1% in the EU countries, while 60.2% in Romania and little more in Moldova – 66.7%. Furthermore, the peculiarity of these diseases suggest a very high risk of disability in population. Indisputably, the aging process is associated with increased risk of cardiovascular diseases, while recent population research estimates 197.9 million of elderly and senile population in Europe by 2025, which makes up 11% and 78.5% higher than in 2010 and 1975, respectively. This fact suggests that situation will get worse.

The incidence of postoperative complications, some lack of their morphological evidence, articles in the third millennium, such as: "The Enigmatic Cardiac Fat Pads: Critical but Underappreciated Neural Regulatory Sites" [1], "Postoperative atrial fibrillation: a billion-dollar problem" [2], "Postoperative Atrial Fibrillation and Mortality: Do the Risks Merit Changes in Clinical Practice?" [3], "The mystery of aortic

dissection: a 250-year evolution" [4], "Atrial fibrillation after cardiac surgery" [5]; contradictory discussions regarding subjects like "Crista aortae ascendentis, ascending aortic fold or Rindfleisch's fold – an enigma" [6, 7, 8, 9, 10]; statistics indicating that cases of postoperative atrial fibrillation have doubled and, in some countries, tripled over each past decade [5, 11, 12] and other facts - all these served as an impetus for the present study.

Material and methods

The study was performed on subjects coming to autopsy not more than 24 hours after death. Their mean age was from 16-week fetuses up to 96 years. A number of research methods have been used: morphometry; histological examination, immunohistochemical method, coloring with Schiff reagent, injectional investigation and anatomical preparation. A total of 354 human aortas were examined: 109 of them were selected for morphometry; 40 - for histological examination, 40 – for coloring with Schiff reagent, 20 – for injectional investigation; 69 mediastinal complexes were subjected to anatomical preparation. The presence of the lymph vessels in the wall of the aorta was confirmed by means of immunohistochemical method.

Results and discussion

The present information is a fragment of a comprehensive study of the morphology of the aorta conducted for the last 12 years. In the presented study, there was determined a wide variability of morphological organization of various portions of the human aorta. There were identified some regularities. Thus, the length of different portions of the aorta depends on the constitutional type of human body: the mean length of the ascending and descending portion is greater in asthenics than in normosthenics and hypersthenics, whereas the longest arch was detected in hyperstenics. The diameter of the aorta increases with aging for both genders, by a mean value of 1.9 mm over each decade.

At readers disposal, there are shown research results on fat body of the ascending aorta (FBAA), size, location (fig. 1) and degree of its development based on gender and age, information which is presented via tables and charts. In 78% of cases, simple bodies are found more frequently than the compound ones, (women - 75%; men - 82.5%). The commonest shape of this fat structure is the strip (in 32.9% of cases: 28.8 - men, 39.4% - women), the rarest form is fold (in 10.8% of cases: 9.6% - men, 12.1% - women). The forms of crest, cylinder and fat pad represent 18.6%, 17.6% and 20% of cases, respectively. Incidence of crests are three times more common in males than in the opposite sex. The strip and fat pad incidence is higher in women than in men by 25% and 13%, respectively (tab. 1).

Regarding the compound bodies, they were found in 22% of the total number of observations, 70.8% - in men; 29.2% - in women. There were detected 10 combinations (tab.2). The most common are presented by strip-cylinder (in 25.0% of cases: 3.7 more frequent in women), followed by cylinder-fat pad and pad-crest (in 16.7%). Requiring notes: it has been observed cylinder-pad is 2.4 times more common in women, and crest-pad has been found only in males (tab. 2). No case of cylinder-fold was deteted. The variability of these compound bodies is larger in men (9 variants) than in women (only 4).

The mean data of morphometric examination of 109 cases of particularities, regarding the length of adipose body and based on gender, differ very little. The average length of FBAA is 3.41 cm in males and 3.43 cm in women.

Up to the age of 35, this index shows no obvious gender difference. It varies in different age periods: the length of the FBAA is the same in women, during 19-35 years and after 66 years; in the second period of adulthood (36-65 years) it is only 2.76 cm; the length of fat body, practically does not vary in men of the first two periods, while group III is smaller. A wide range of minimum and maximum values is observed in men: in the Ist group, the maximum value exceeds 3.75 times the minimum; in the IInd group – 7 times; group three - 5.3 times.

Table 1

Simple FBAA incidence in men and women (%)

Shape	str	cl	cr	fl	fp
Men	28.8%	19.2%	26.9%	9.6%	15.4%
Women	39.4%	18.2%	9.1%	12.1%	21.2%
Mean Incidence	32.9%	18.6%	20%	10.6%	17.6%

Note: str – strip; cl – cylinder; cr – crest; fl – fold; fp – fat pad.

Table 2

Incidence of compound FBAA (%)

	str/cl	str/cr	str/fl	str/fp	cl/fp	cl/cr	cr/fp	fl/fp	str/cl/fl	str/cl/fp
Men	11.8	5.9	17.6	5.9	11.8	5.9	23.5	0	5.9	5.9
Women	42.8	14.3	0	0	28.6	0	0	14.3	0	0
Mean Incidence	25.0	8.3	12.5	4.2	16.7	4.2	16.7	4.2	4.2	4.2

Note: str/cl – strip-cylinder; str/cr – strip-crest; str/fl – strip-fold; str/fp – strip-fat pad; cl/fp – cylinder-fat pad; cl/cr – cylinder-crest; crest/fat pad; fold-fat pad; str/cl/fl – strip-cylinder-fold; str/cl/fp – strip-cylinder-fat pad.

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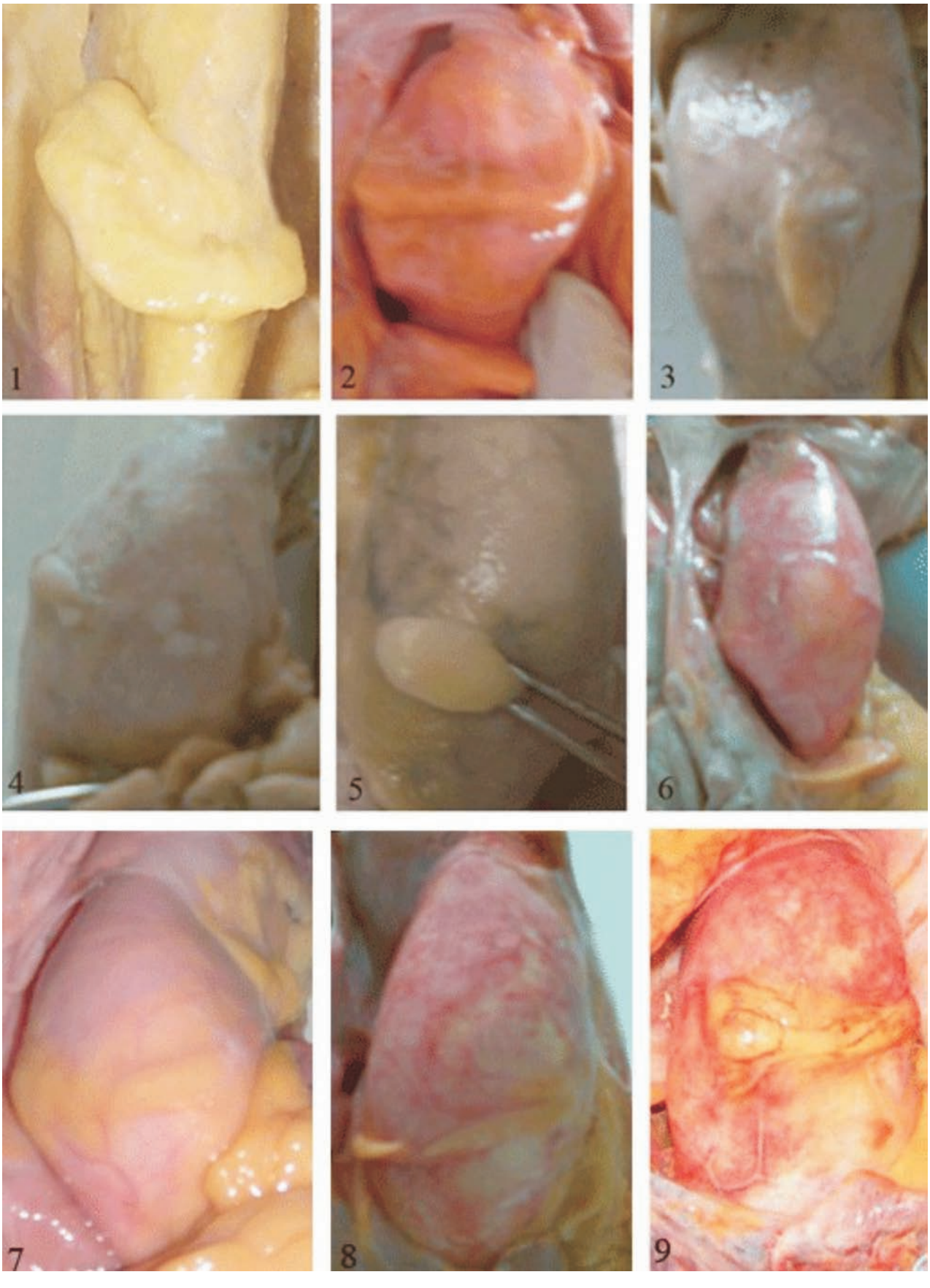

Fig. 1. Variability of the ascending aorta fat pads. 1 – fold, 2 – horizontal crest, 3 – vertical crest, 4 – fragmented fat pad, 5 – fat pad on the posterior surface of the ascending aorta, 6 – fat pad on the right surface of the ascending aorta, 7 – bundle, 8 – bundle-fold, 9 – bundle-pad.

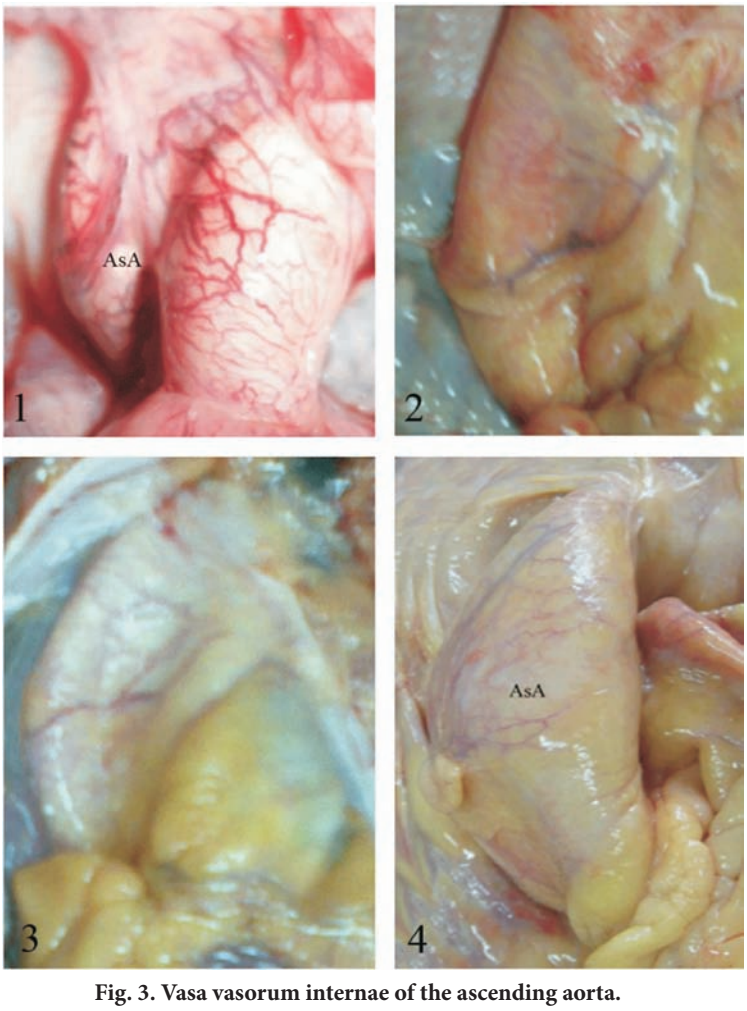

**Fig. 3. Vasa vasorum internae of the ascending aorta.
1 – Ascending aorta and pulmonary trunk of the fetus of 29 weeks,
2 – Woman of 50 years, 3 – Man of 61 years, 4 – Man of 59 years.**

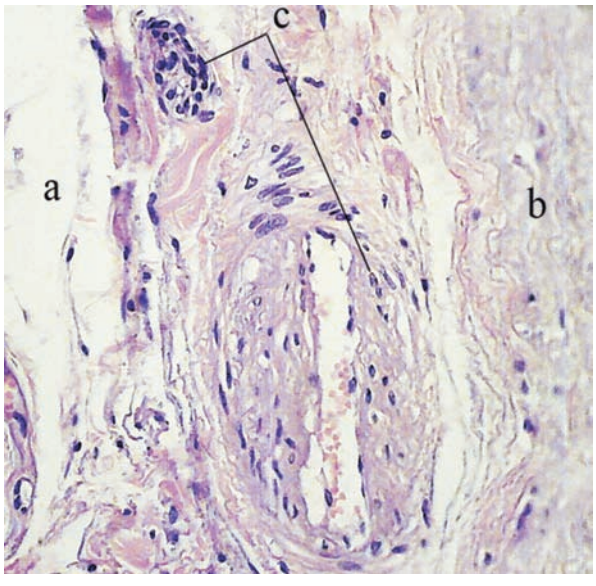

**Fig. 5. Glomic cells of the ascending aorta
in deep layer of the adventitia. a – adventitia,
b – aortic media, c – glomus cells.**

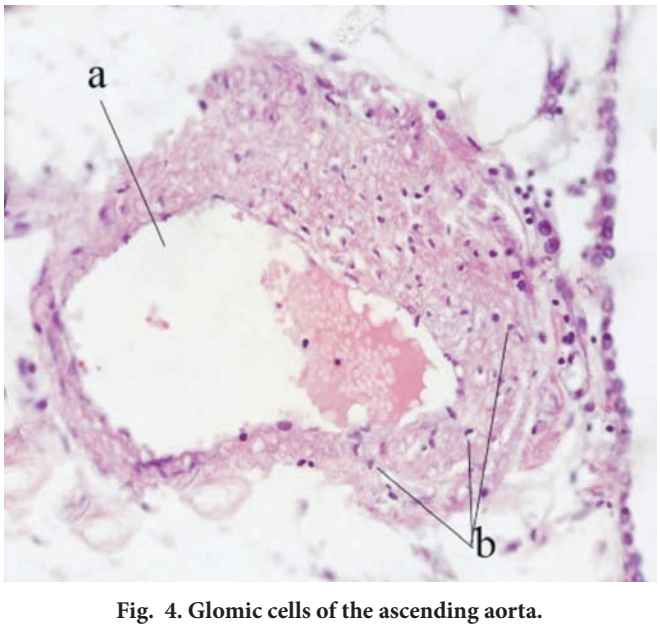

**Fig. 4. Glomic cells of the ascending aorta.
a – vein, b – glomus cells.**

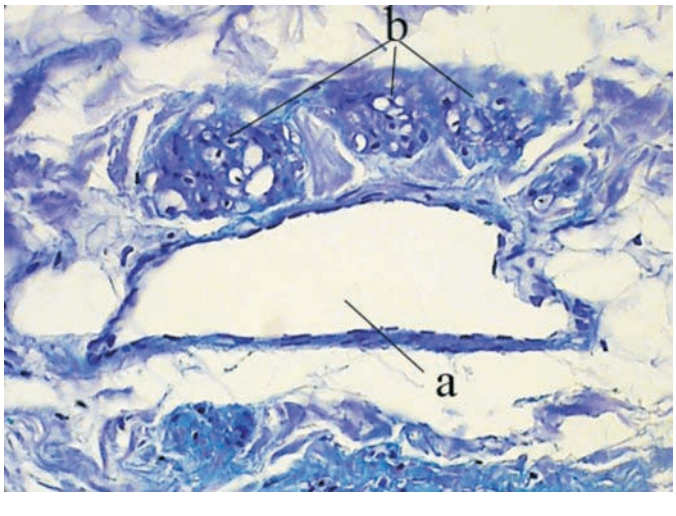

**Fig. 6. Glomic cells of the ascending aorta.
a – lymph vessel, b – glomus cells.**

The biggest difference in the opposite gender representatives was found in group II - 6.8 times, in group III - 4.6 times, and the Ist – only 1.2 times.

Even more interesting data are obtained through analysis of the length of the fat body of ascending aorta in different age decades (fig. 2). Up to the age of 30 there is no significant difference in FBAA length regarding gender. Subsequently, by the age of 40-41, a sudden increase occurs in males, while a decrease is observed over the next decade; ultimately, a stagnation occurs up to the age of 60-61. Subsequently, a sudden decrease may come.

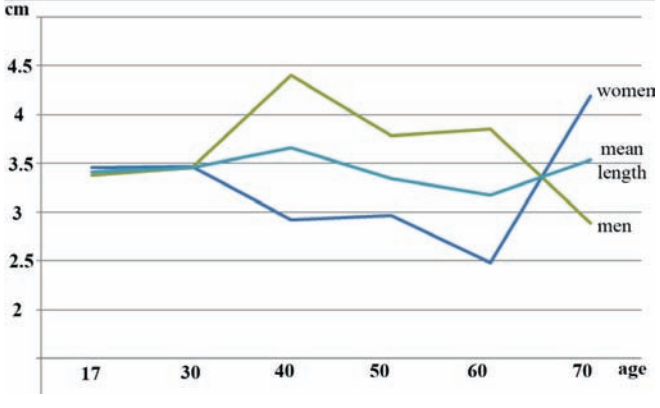

Fig. 2. The mean length of FBAA.

In women aged over 40, the dynamics of fat body size occurs directly opposite: the decrease- up to 50 years old; stagnation - up to 60; sudden decrease - untill 61 is followed by a sharp increase.

At the age of 65, FBAA length values are equivalent in both genders, whereas the index continues to rise to its maximum in women, and decrease until the minimum index in men.

The highest values of ascending aorta fat body length (7.0 cm) were found in males aged from 52 to 65, both deaths occured due to cardiovascular disease. By the way, the other cases which were observed (length more than 6.5 cm), also belong to the deceased men aged 52, 54 and 57 years, with similar causes of death. The maximum indices were recorded in females aged 57 and 77 years old, deceased as a result of myocardial infarction and acute cardiovascular disease.

Hence, the persons do not differ either in age or diagnosis. All of them suffered from right atrial hypertrophy.

It is obvious that, the level of development of the aortic fat body does not correspond to the development of the adipose tissue in general. We have observed cases when the aortic fat bodies are well-developed in cachexia, and vice versa, less-developed in obese people.

Most common FBAA starts from the anterior aortopulmonary groove and continues on the anterior and right sides of AA's, especially in women (in 39.5% vs. 31% in men). Then, it is followed by the location of fat body mentioned only on the right side of ascending aorta (in 16.6% ; 1.4 times more common in males); on the anterior and right sides (in 12.8% ; 3.2 times more common in men). A larger fat body which made up 60%, and in some cases more, out of the AA's circumferential length was found in 2 cases, more common in males. The location of FBAA only on the posterior surface was recorded only in males (in 4.2% of cases), and only on the anterior part and in fragmented form (the location on anterior and posterior surfaces) - only in women (in 7.9% and 2.6% of the cases, respectively).

Table 3 demonstrates conclusively the lack of interdependence between the development degree of the AA's fat body and overall fat body. Regarding the development of FBAA degree, extreme variations were detected: poorly-developed adipose bodies were found in men, and well or very well-developed degree predominantly in the opposite sex.

Regarding the above mentioned we can conclude that the location of FBAA is determined by the shape of the right auricle and the degree of development depends upon the force of contraction of the right atrium.

The very shape of the crest dominance and the combinations including it in men and strip and fat pads in women indicates the interdependence between formation of specific type of FBAA and atrial contraction power. In determining the fat body appearance both the auricle shape and the configuration of its contact line with the ascending aorta are important.

What is the most primary: the formation of vasa vasorum internae (VVI) of fat body area or fat body? Figure 3.1 shows that, in absence of fat deposits during the prenatal development, VVI direct towards the common location of the fat body of the ascending aorta. In adults, the fat body is formed and can be seen with naked eye, VVI are directed to the fat body, regardless of its location: on the anterior surface (fig. 3.2), on the lateral (fig. 3.3) or posterior (fig. 3.4). Since, everything is a logical part of nature, this can be only explained by the existence of functionally important structures at this level, which requires a rich vascularization, while the adipose tissue performs auxiliary functions, more likely those of supporting and buffering. Our microscopic study shows that the vasa vasorum internae ensure the vascularization of the structures

Table 3

Degree of development of the fat tissue

	Very low		Low		Moderate		High		Very high	
	FBAA	GFT	FBAA	GFT	FBAA	GFT	FBAA	GFT	FBAA	GFT
Women	0%	2.5%	32.5%	7.5%	15%	52.5%	30.0%	25.0%	22.5%	12.5%
Men	4.3%	1.4%	17.4%	7.2%	11.6%	46.4%	39.1	29.0%	27.5%	15.9%
Mean	2.8%	0.9%	22.90%	7.3%	12.8%	48.6%	35.8%	26.6%	25.7%	27.5%

Note: FBAA – fat body of ascending aorta; GFT – general fat tissue.

similar to the carotid corpuscle that are located within the fat body of the ascending aorta.

It is natural, that the adipose tissue, while diminishing the systolic shocks of atrial auricles and the pulsating ones during the contractions of the left ventricle, provides optimal conditions for the structures involved in regulation of the blood circulatory system.

Glomus cells were found within the fat body of ascending aorta, along the arterial vessels, venous and lymphatic. They were recorded at different depths of aortic adventitia from subepicardic layer (fig. 4) until to the limit of the aortic media (fig. 5). Hence, the glomus structures, which are located in fat body of the ascending aorta, widely vary in shape, size, depth of location and their relation to blood and lymph vessels (fig. 4, 5, 6).

In most cases, glomus cells represent clusters, forming corpuscles. As a rule, the smaller ones are located deeper, near the aortic media. The size of these structures varies from 100 mkm to 2 mm. Along with age, the pseudocapsule becomes more defined (fig. 4), the number of supporting cells also increases.

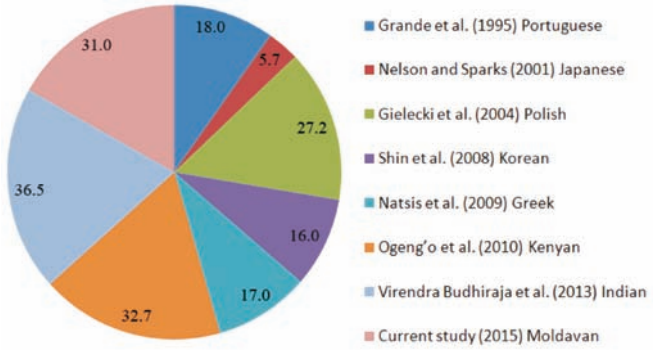

Fig. 8. Frequency of variant branching of aortic arch in different populations (%).

The obtained results prove the permanent presence of multiple rounded or oval lobulated structures with specific sources of vascularization (vasa vasorum internae) inside the ascending aorta fat body. The number and size of lobules are highly variable. The lobules are surrounded by connective fine capsules, containing a variable number of glomus cells. It would not make sense to mention these obvious facts if not applied clinical importance of this fat accumulation and a close relationship between its form and the nature of the vascular net.

The presence of these structures at all ages, specific sources of their vascularization like vasa vasorum internae with origin on the concave side of the ascending aorta, normally 1-1,5 cm above FBAA, which coincides with the location of the reflexogenic area, is described by Iu. C. Comroe [13] in his experimental study; it is reasonable to conclude that reflexogenic area is localized in the fat body of the ascending aorta.

The trajectory of the large lymph collector providing drainage from the sinus node area was described on the base of our mesoscopic study with the use of the Schiff reagent. Histologic and immunohistologic methods allowed us to reveal lymph vessels and nodules in the aortic adventitia.

Studying all the nervous, vascular and lymphatic elements of the fat body of ascending aorta, we came to the conclusion that disruption of the above named collector during the surgical intervention leads to the development of atrial fibrillation in the postoperative period. While studying all divisions of the thoracic aorta we have not managed to reveal such combination of specific features of vascular, lymphatic and nervous apparatus as in the area of FBAA. These observations suggest this anatomical structure to be a functionally important area of the aorta.

Based on a substantial material, accumulated over several years of research, we tried to answer a series of questions which refer to the functional morphology of the ascending aorta, especially of its fat body. Simultaneously, we cannot affirm that the so-called "enigmatic ascending aorta" reported by many clinicians over the last decade, has exposed all its secrets. We revealed typical for endocrine gland tissue and some neurovascular complexes in its fat body contents. What is their function? Such information has not been found in available literature.

Our study shows a lot of anatomical variants of aortic arch. Three shapes of the aortic arch were asserted: roman arch - in 31 of 42 cases (73.6%), gothic arch - in 10 cases (24%) and crenellated arch in one case (2.4%). The atherosclerotic damage in the last two shapes is more pronounced.

The typical ramification into three branches was detected only in 69.05% of cases. The greatest variability of the aorta branching occurs in normosthenics (fig.7). The variants were found in 13 out of 42 cases (30.95%), whereas, 2 branches - in 9.5%; 4 - 19.04%; 5-2.41%. There is a difference compared to other studies of the authors from various countries (fig. 8).

In surgical plasty of aortic arch, postoperative complications appear, such as: damage of the vagus, recurrent laryngeal and/or phrenic nerves, sometimes lymph thoracic duct [14, 15]. There are few publications on morphology regarding this issue, which can be explained by the fact that these complications are not fatal for patients. The actual problem may occur, if the patient has pre-operative respiratory disorders which can worsen the prognosis. The incidence of the above mentioned nerve damage and thoracic duct is not high, but tends to increase, which is explained by the increased incidence of surgical interventions on the organs which are sintopically related to the above-mentioned anatomical formations. Complete information is required with reference to the variability of aorta syntopy and adjacent nerves [16, 17].

The larynx is involved in actions of swallowing, breathing, coughing and phonation. These functions are dependent upon the normal movements of the vocal cords that are controlled by muscles innervated by branches of recurrent laryngeal nerves.

According to the statistics, the recurrent laryngeal nerve palsy is caused by tumors in 1/3 of cases, in 1/3 of the cases – by the trauma and 1/3 of cases – by unknown causes (idiopathic).

The approximate length of the left recurrent laryngeal nerve is 12 cm, whereas the right is only about 6 cm. This distinction explains the fact that the most common subjected to

trauma is the left recurrent laryngeal nerve in the chest region. This nerve damage occurs in 11-32% of thoracic surgery. Most publications regarding traumas to this nerve are performed by otolaryngologists who deal with nerve injury on thyroid surgical interventions. Within the chest, the left recurrent nerve shows a close contact with the aorta, the trachea, the left atrium, left main bronchus, esophagus and lymph nodes.

The recurrent left laryngeal nerve, which passes under the arch of the aorta, is placed in tracheoesophageal fascia, in the tracheoesophageal groove above or slightly anteriorly of the above-mentioned groove. Therefore, not only the nerve is at risk during surgery on the aortic arch, but also esophagus, trachea [18]. The damage of the inferior laryngeal nerve leads to the establishment of the respective vocal cord in para-median position; there is a risk of aspiration of heterogeneous bodies into the trachea.

As a rule, the aortic arch plastic surgery, mediastinoscopy, intrapleural analgesia, left lung lobectomy, lymphadenectomy are associated with various complications depending on the individual syntopic peculiarities of the superior mediastinum organs.

The damage of phrenic nerves, vagus and left recurrent laryngeal one and others, arise discussions among clinicians [19-31]. This problem is, usually, referred to morphological changes in some patients. It still has not found the right place in specialized literature. Hence, the knowledge about individual features can help reduce the risk of damage to adjacent organs.

Commonly, the term „aortic arch" stands for a segment of the aorta, which branches off to the head, cervix and upper limbs. According to our investigation results, the aortic arch length ranges from 2.7 cm to 6 cm. We have established an interdependence of body constitution type and length of the arc, but not between its length and number of branches which ramify from the aortic arch, although these individual variations can be frequently seen.

Syntopical relations of the aortic arch, largely correlate with its structure. The aortic arch may be slightly (roman arch) or sharply curved (gothic arch). In the first case the arch branches are more evenly spread, whereas in the second case they start from the shortest upward or downward segment.

The nerves and adjacent vessels correlations and paths vary greatly in different people. We have paid particular attention to the essential moments of faster exposure of nerves and vessels during surgery. Such an approach helps to prevent their trauma, if it occurred – in selecting appropriate neurosurgical technique in neurorrhaphy.

Our study showed variants of aortic arch branching and insertion of arterial ligament, atypical left vagus and recurrent nerve tracts, the existence of the supernumerary trunks of the latter and thoracic duct lymph.

In typical cases, left vagus nerve intersects the upper margin of the aortic arch, placing itself in front of the left subclavian artery, although in 28.57% of cases it passes laterally of this vessel, of which half is 1 - 1.5 cm to the left. The most common lateral placement of the left vagus nerve (LVN) is observed in hypersthenics (50%), in asthenics it is registered in 44.5% of the cases, and in normosthenics – 22.7% of cases.

In 8.16% of cases, LVN passes medially from LSCA at a distance of 3-4 cm. Hypersthenics make up 50% of cases; normosthenics and asthenics the other 50%, 25% respectively for each of the cases.

By the way, the constitutional type was determined by the ratio between the waist and chest perimeter (at the level of the X rib). People with a coefficient up to 0.5 were attributed to asthenics; those within 0.51 to 0.60 to normosthenics; 0.61 and more to hypersthenics.

The inferior edge of the aortic arch is intersected by LVN at a distance of 0.2-1.3 cm laterally from the aortic end of the arterial ligament. In 80% of cases the left vagus passes anteriorly to the aortic isthmus, in 15% of cases - on its medial edge, in 5% of cases – on the lateral edge respectively.

The aortic arch is surrounded all over by the cardiac plexus formed by branches of the sympathetic trunks and of vagus nerves. In the forefront, the aortic arch is covered by the pleura, the anterior edges of the lungs and thymus, except for the middle portion of the arch, which is not covered by the pleura, thus allowing to perform the retrosternal anesthesia of the cardiac plexus. The left phrenic nerve is located anteriormedially to the left vagus nerve. The left intercostal supreme vein passes between these two nerves, which have an oblique direction to the anterosuperior part.

Inferior to the aortic arch, there are 4-6 lymph nodes sized from 0.5-1.0 cm, left pulmonary artery and the left bronchus. The aortic arch intersects them and extends to its descending part. The arterial ligament is located on the anteroinferior half-circumference of the aortic arch. Posteriorly from the arch of the aorta, from right to left, there are the trachea, esophagus, lymphatic thoracic duct.

The brachiocephalic trunk, left common carotid artery and left subclavian artery take their onset from the upper side of the aortic arch to the cranial direction. The incipient portion of the superior vena cava is located toward the right.

The classical description of the correlation between the aortic arch and the structures located on its posterior part is as follows. The posterior part of the aortic arch comes in contact with the anterior wall of the trachea. The esophagus is located toward the left, at the crossing arch of the descending aorta. The recurrent nerve passes through the anterior tracheoesophageal groove, whereas the lymphatic thoracic duct – through the left edge of the esophagus. The recurrent laryngeal nerve contains myelinated fibers. These fibers are placed anteriorly within the vagus nerve.

The recurrent laryngeal nerve fibers begin to rotate medially along the vagus nerve, until are separated from the vagus nerve respectively [19]. The level of separation takes place according to the concave edge of the aortic arch in 67.4% of cases, 15% - in the upper portion of the ventral surface of the arch, in 17.6% of cases - in its lower portion. In most cases, the left recurrent laryngeal nerve (LRLN) passes from the left arterial ligament, being closely allied to the latter; the divergence angle between LRLN and LVN ranging from 20° to 85° degrees. The existence of the accessory left recurrent laryngeal nerve (ALRLN) takes place in 8.16% of cases, in

3/4 of them - to hypersthenics; in ¼ of cases - to normosthenics and in no case to asthenics. In 50% of cases, ALRLN was detected in association of the aortic arch with three branches, the other 50% - with 4 branches. As it was already mentioned above, in common cases, LRLN is placed in the anterior tracheoesophageal groove or in the forefront of it. The accessory trunk is located toward the front of the trachea in all cases. We found no dependence of this variant neither to the arch length or to type of body constitution. Therefore, surgeons should pay special attention during interventions to hypersthenic patients and occurrence of accessory branches originating from the aortic arch, which can result in reduced cases of recurrent laryngeal nerve trauma and the follow-up consequences that can occur.

The path variations of the left recurrent laryngeal nerve are not common. To alleviate any postoperative complications, cardiothoracic surgeons must know the possible variants. There were cases, where this medial nerve was crossed by the ligamentum arteriosum [32].

Clinicians highlight three areas related to the risk of left recurrent nerve injury:

A. The high risk area of direct injury – toward the front part of the inferior portion of the trachea left wall;

B. The high risk area of indirect injury, induced by compression - between the inferior part of the trachea wall and aorta;

C. Low risk area - along the right wall and in the forefront of the upper portion of the anterior wall of the trachea.

Syntopic relations of the recurrent nerve, as well shows practical interest. In 12% of cases, the left recurrent nerve starts from the ventromedial area of the vagus nerve; in 88% - from the dorsomedial area. Inside the chest, left recurrent nerve is closely allied to the aorta, trachea, left atrium, left main bronchus and esophagus. Under the arch of the aorta, posterior to the Gross triangle (an area limited anteriorly by the phrenic nerve, posteriorly – by the vagus nerve and inferiorly - by left pulmonary artery), the recurrent nerve comes in contact with 3-5 lymph nodes ranging from 0.5 to 1.0 cm. In case if the lymph increases, the nerve becomes essentially flattened, that is 2-3 times thinner (also much wider) compared to the normal state, causing phonetic disorders which are difficult to diagnose.

In 10% of anatomical specimens that have been examined, the recurrent nerve was represented by two or three trunks. According to data of C. Weeks, J. Hinton (1942), this phenomenon is found more frequently - in 78% of cases [33]. The nerve trunks are always distributed toward the frontal part. At the posterior part of the aortic arch at the level of the concave portion, the distance between trunks varies from 2 to 5 mm, but on the convex surface, it increases to 10-14 mm. Some sources describe recurrent laryngeal nerve splitting into two branches (medial and lateral) at the lower limit of the larynx. Probably the inferior division explains the presence of several of the recurrent nerve trunks. In case of supernumerary trunks, one is placed in the anterior tracheoesophageal groove, others - on the front part of the trachea. The fact that all identified additional nerve trunks have been found in humans of hypersthenic body type is of interest.

There is a uniformity: supernumerary trunks of the left recurrent nerve are detected in cases when the left vagus intersects the convex margin of the aortic arch near its origin or at the level of the brachiocephalic trunk. Thus, based on intraoperative viewing of the vagus nerve, we can detect the location of the left recurrent nerve and the existence of accessory nerve, which, in turn, will reduce the risk of their injury and therefore prevent the paralysis of the vocal folds.

According to our observations, the typical location of the left recurrent nerve, in the tracheoesophageal groove, occurs only in 61% of cases. In 39%, it goes 3-10 mm medially from the groove, on the anterior surface of the trachea.

There are other unclear issues which refer to the impact of the aorta morphological organization on the potential exposure of this vessel to various diseases. Although there are various hypotheses of the pathogenesis of atherosclerosis (lipid, response to aggression and to initial, unified, neurogenic, infectious atherosclerotic lesions), yet the risks are known: the gender and genetic factors, age, bad diet, obesity, pollution of air, sedentary lifestyle and others [33]. Taking into account our observations, we can say that other factors also cannot be excluded, like lymphatic drainage and structural features of aorta which affect the hemodynamics of this major vessel.

The study of over 300 intact human aortas, showed their diversity by different criteria, especially in length and diameter. Thoracic and abdominal portions vary both in length as well as in their correlation. A similar situation is asserted at incipient and terminal portions of the lumen of the descending aorta. Under equal conditions (gender, age, constitutional type), the bigger the coefficient values are, the more advanced are the manifestations of atherosclerosis – both extension, as well as its degree of severity. We refer to the length coefficient. It makes up the ratio between the length of the descending thoracic aorta and of abdominal aorta. Lumen value is the ratio between the lumen of the descending aorta in its incipient portion (upper chest) and of the terminal portion (lower abdominal).

The above-mentioned indices are not the major determinants of atherosclerosis onset and pathogenesis, but both have impact on the aortic hemodynamics. It should be noted that, while examining various morphological aspects, we should as well consider the conditions of the hemodynamics.

A difficulty which arose during our research, just at the early stage of studying the specialized literature referred to terminological differences in publications on the topic. The terms used in describing portions of the aorta and its components at the macroscopic and microscopic level are very variable. F. Unger and et al., described the need to improve terminology of adipose bodies of the aorta [34]. We studied several aspects of aortic morphology and consider the problem of terminology as an acute one which can affect people's lives.

In order to avoid double or even triple interpretation of the presented data, we believe that we need to clarify the used terms. The authors unanimously use the term of "ascending aorta" in describing the aorta, although this notion defines different meanings. According to some data (Clinical Anatomy Associates, 2015), the ascending aorta ends on imaginary

Table 4

Terms used for fat body of ascending aorta

Authors	Terms
E. Rindfleisch (1884)	Semilunar fold, ridge, vincula
Z. Davis. (1927)	Periaortal fat body
K. Smetana (1930)	Fat ring
H. F. Robertson (1930)	Periaortal fat pad
W.W. Parke, N.A. Michels (1966)	Aortic ridge
G. T. Lebona (1991)	Ascending aortic fold
L. Gross (1921), Z. Davis (1927), F. Unger, W.Gerald Rainer (1999), George Falkowski, Ilya Dzigivker, Dani Bitran (2001)	Transverse fold
Zev Davis, H. Kurt (2000, 2004)	Aortic fat pad
G. T. Lebona (1993)	Ascending aortic folds: oblique, horizontal, vertical, oval, horizontal-oblique, vertical-oblique, vertical-horizontal, vertical-horizontal-oblique, oval, oblique.
J. J. Morrison, M. Codispoti, C. Campanella (2003)	Transverse ridge
Felix Unger (2005), J. J. Morrison (2003)	Fold, crest

horizontal line that passes through the sternal Louis angle, whereas aortic arch continues. This is an important anatomic landmark. Often, surgeons use an oblique planning which passes from the upper limit of the ascending aorta to the proximal point of the brachiocephalic trunk origin. This landmark is useful in surgery, whereas it is not relevant to anatomy.

Louis angle also indicates the upper edge level of the pericardial sac. Thus, it turns out that the ascending aorta is the intrapericardial portion of the aorta in surgery too and that the pericardial sac may serve as effective anatomic landmark for the separation of the ascending aorta from the aortic arch.

Other authors consider the ascending aorta as a portion of the aorta from the bulb-tubular junction to the origin of the brachiocephalic trunk. There is the opinion that the ascending aorta is a vessel segment which passes from the ventriculo-aortic junction to the first branch of the aortic arch.

From the above explained, we may conclude that the used terms are not well-defined. The divergence of views in cases where authors do not indicate their option, can lead to misperception of that information.

This research paper uses the term of „ascending aorta" as part of the aorta from the aortic valve to the emergence of the brachiocephalic trunk, which, in turn, consists of two segments: the bulb and the tubular portion. The aortic arch is the segment of the aorta passing from the base of the first branch to the aortic isthmus, while the descending thoracic aorta – from the isthmus to the diaphragm, the abdominal aorta – under the diaphragm.

Some formations, such as subepicardic fat bodies, are very variable, and have from 13 to 15 names per each, but none of them fully reflects the location, shape and their function (tab. 4). In other cases, one and the same term is applied to different structures, e.g., "anterior fat body"- placed between the aorta and pulmonary trunk [35-38], for the one placed on the ventral side of the atria [39] between the aorta and right pulmonary vein.

In order to avoid confusion in terminology and prevent misperception of the information by the readers, I have used and recommended a term which does not lead to confusion of concepts and structures. As it is in case of multiple subepicardic adipose bodies. Thus, for the adipose collection of the ascending aorta which comes in contact with the edge of auricle of the right atrium, instead of various terms used to define the very structure: "semilunar fold, ridge, vincula, anterior fat body of the AA, periaortic fat body, transverse aortic fold, the ascending aortic fold" and others. As none of the names mentioned fully reflects the location or shape of the anatomical entity of a particular importance at present, we suggest to call it "Rindfleisch' fat pad" (RFB), showing a particular respect to the morphologist, who in 1884, was first to present the adipose subepicardic structure associated with the ascending aorta.

While physiologists are clarifying if intramural structures of the ascending aorta similar to the carotid corpuscle are ganglia, paraganglia or endocrine gland, chromaffin or non-chromaffin and which system they are related to (sympathetic or parasympathetic) we used such neutral terms as corpuscle or glomus.

Conclusions

1. Fat body of the ascending aorta is variable in form, location and individual size. It represents a formation of physiological importance, includes many glomus-type structures and often – lymph collector from the sinus node area. Obtained knowledge can be implemented in selecting a mini-access in surgery on this zone of the aorta.

2. The variation of the syntopic relationships between the arch of the aorta and left vagus and recurrent nerves is much more diverse than it is expected, a fact which should be taken into account while performing surgical interventions on the superior mediastinum.

3. Currently, when surgical maneuvers on the aorta have become routine, updating of terminology of aortic morphology has become an urgent necessity.

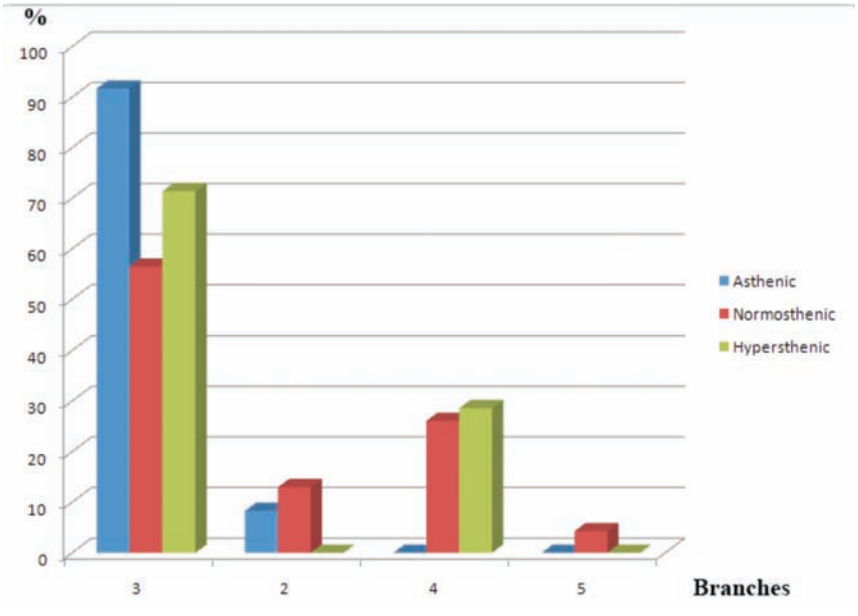

Fig. 7. Frequency of different branching patterns of the aortic arch.

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Curierul medical, April 2016, Vol. 59, No 2

Clinico-morphological manifestations of atherosclerotic lesions of cerebral basilar arteries

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Abstract

Background: Endothelial dysfunction is an early sign of atherosclerosis, which favors the increase of vascular permeability, activation of mast cells and migration of leukocytes, lymphocytes, macrophages, platelet adhesion, smooth muscle cell proliferation and eventual vasospasm, which together determine a proinflammatory status. Angiogenesis is an important pathogenic element of atherosclerosis in stages of complicated plaques, along with mast cells and macrophages.

Histotopographical analysis of the distribution of newly-formed vessels as a feature of angiogenesis, expression of mast cell and macrophage in different types of plaques in different arterial vessels in patients with atherosclerosis and metabolic syndrome complicated by atherosclerosis.

Material and methods: We studied 34 patients with cerebral artery atherosclerosis. To determine the expression of mast cells in the affected vessels, we used anti-MCT. Macrophages were identified using specific marker CD-68 and newly-formed vessels respectively through the application of CD-105 (endoglin).

Results: Assessment of the results was based on the final determination of the density and intensity of reaction, as reflected in the quantitative ratio of the different areas of atheromatous plaques. Stained positive mast cells, macrophages and newly-formed vessels have been found in several types of atherosclerotic plaques, especially in adventitia and in the immediate vicinity of the plaques and subendothelial layers. We found a statistical correlation between the type of plaque and clinical data.

Conclusions: Immunohistochemical method is efficient for the determination of mast cells, macrophages and newly-formed vessels of atherosclerotic plaques, directly reflecting many important pathogenic factors of atherogenesis in patients with atherosclerosis of the brain arteries.

Key words: atherosclerosis, angiogenesis, mast cells, macrophages.

Introduction

One finds it hard to nominate another disease like atherosclerosis, on the pathogenesis of which there exists such a large number of theories, hypotheses, assumptions and even speculations. For understanding the pathogenesis of atherosclerosis, precise knowledge of ways and mechanisms that determine the penetration of atherogenic lipoproteins in the vascular wall is of major importance. An equally major role is assigned to the analysis of protection and adjustment reactions, which condition the metabolism of the vascular wall in normal circumstances and in atherosclerosis. For a deeper understanding of mechanisms that set off in vascular wall in atherosclerosis, it is necessary to conduct a detailed, structural-functional study of cellular elements of the artery wall, their participation in lipids uptake and synthesis of collagen and elastin. Knowing the vascular wall metabolism in atherosclerosis, defines, to a wide extent, the ways of preventing and treating this disease.

We believe that atherosclerosis is the primary cause for developing ischemic heart disease, stroke, arterial lesions of lower limbs and other organs. Atherosclerosis is a disease of the XXI century, which turns more into a burden or a cross that mankind carries as punishment for current civilization. One gets the impression that, by this disease, God has punished people for their compulsion towards social conflicts, limitation of active lifestyle, excess of food and sleep and many other bad habits.

The clinical features of atherosclerosis depend largely on the lesion position in the vascular ramification. The analysis of the sectioned sample shows that, sometimes, it is enough to have in the coronary heart artery or cerebral basilar artery an atherosclerotic plaque in a certain location to cause extensive transmural myocardial infarction or vascular changes in the brain (ischemic or hemorrhagic strokes) [1].

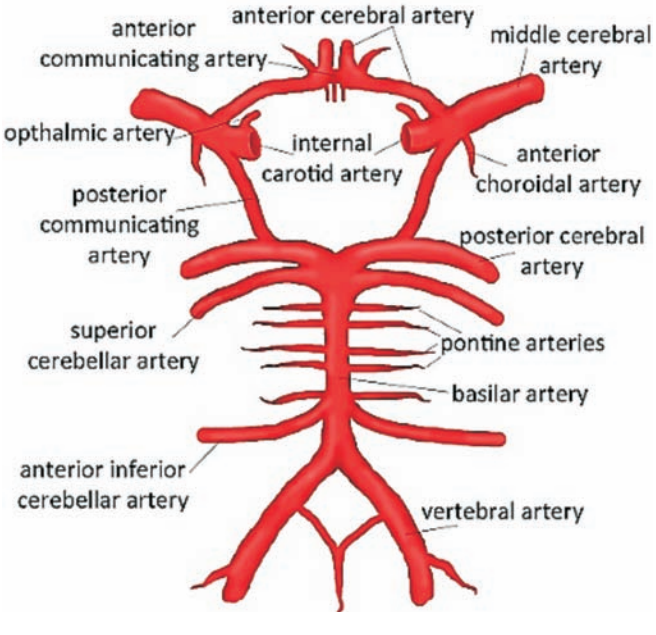

Fig. 1. Circle of Willis.

Atherosclerosis primarily affects elastic and muscular elastic arteries. Cerebral arteries lack external elastic lamina, and the internal elastic lamina is well developed. The arteries of the Circle of Willis (fig. 1) are muscular arteries, as their middle layer is well developed.

Strokes can occur at any age and in any gender. To a large extent, they reflect the degree of impairment of cerebral vessels (carotid, basilar and cerebral arteries). Figure 2 shows the normal structure of the inner layer of the basilar artery in scanning electron microscopy.

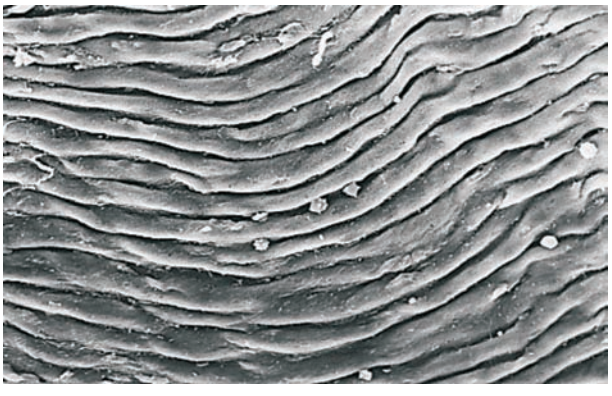

Fig. 2. Scanning electron microscopy of the surface of normal basilar artery (x 3200).

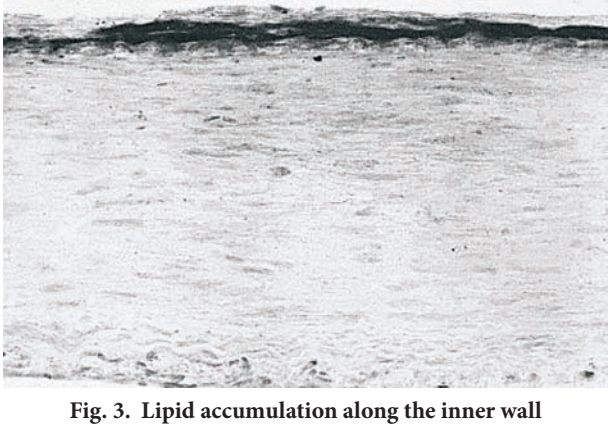

Fig. 3. Lipid accumulation along the inner wall of basilar artery elastic lamina, patient's age – 37 years old (Oil Red staining, x 460).

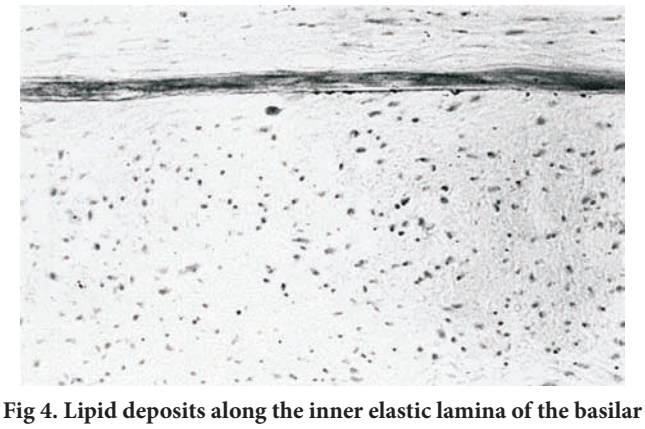

Fig 4. Lipid deposits along the inner elastic lamina of the basilar artery, patient's age – 67 years old (Oil Red staining, x 460).

In lipid spots from the basilar artery of the brain, lipid deposits in the ground substance are observed mainly in the surface layers of the internal tissue, in the internal elastic lamina (fig. 3, 4). At this stage of lipidosis, the elastic lamina keeps its tinctorial properties and is intensely fuchsin-stained by Weigert method.

In further development of lipidosis of the cerebral basilar artery, significant lipid depositions are observed not only in the internal elastic lamina, but also on its loose fibers. Sometimes, the internal tissue lipidosis is diffuse; lipids are deposited on the internal elastic lamina, on its plaques, which are separated and on tissue layers between them.

Without making a comparison with similar changes in other arteries, in incipient forms of atherosclerosis of cerebral basilar artery, one can get the impression of primary isolated adipose degeneration of the internal elastic lamina in atherosclerosis and only a secondary lipid deposition in the intima. The study of characteristics of lipid deposition in different arterial systems, as well as in various segments of the cerebral basilar artery, makes it possible to determine the stage of lipid deposition on the surface of elastic membranes or in cell elements [2].

Degenerative changes in the internal elastic lamina of the cerebral basilar artery differ from the changes of elastic elements of other arteries investigated by us, which can probably be explained by significant selective lipid deposition on the internal elastic lamina that becomes adipose and simultaneously thickened. In this case, one does not observe primarily the lysis, but the tumefaction and the cleavage of the membrane. On fuchsin staining, such a membrane has a dark violet color and loses its double refrangibility (refractive power) in polarized light.

At lipid deposition and subsequent impregnation in the aorta and coronary arteries of the heart, signs of degenerative changes are also found in intima elastic structures. These changes occur not only by changing their tinctorial properties, but also by adhesion, evening and fragmentation of some fibers. Contours and boundaries of elastic fibers become blurred; in some sites, thin elastic fibers are subject to lysis, as if dissolved in the surrounding lipid mass. At the same time, one often observes the alternation of areas of lysed elastic fibers with areas where their destruction is just beginning, while their tinctorial properties are maintained.

In progressive forms of atherosclerosis, the vascular wall metabolism is directed to the use of excessive amounts of emerged lipids and, thus, is connected to the discharge in lipidosis portions of various cellular elements from the changed sites of the arterial wall (predominantly smooth muscle cells) and mobile cellular elements (mononuclear monocytes) from the bloodstream, as well as to the activation of oxidative-regenerative and hydrolytic enzymes.

This process is accompanied by formation of new connective tissue, a phenomenon which inevitably leads to production by the mesenchymal cells in situ of sulfated fractions of glycosaminoglycans, in particular dermatan sulfate. Probably, in the early stages, this reaction can be viewed as a protective-adaptive one.

The foam cells accumulate a large number of cholesterol compounds and fatty acids. In the process of absorption of lipids by the cells and formation of foam cells, the latter, due to reactive changes, are surrounded by immature connective tissue, which, in maturation, forms layers that separate foam cells from their surface layer and the conjunctive tissue.

Alongside with lipid accumulation in foam cells and connective tissue growth, one observes marked degenerative

changes of elastic fibers that also comprise the internal elastic lamina. On accumulation of lipids in the basilar artery inner coat, basophilia and metachromacia of the internal elastic lamina are especially evident. Lamina „dissolves" gradually, which results in its penetration by the lipids (fig. 5).

Fig. 5. Internal elastic lamina lysis. Lipids penetration in basilar artery middle coat and atheroma formation in the media under internal elastic lamina defect (Oil Red staining, x 460).

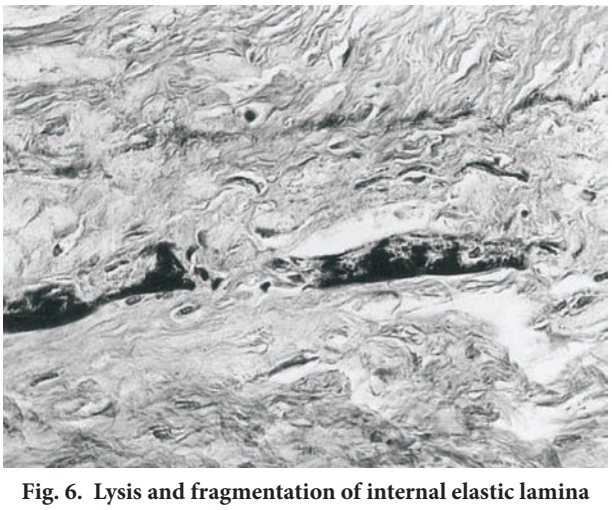

Fig. 6. Lysis and fragmentation of internal elastic lamina in the site of atherosclerotic plaque in the basilar artery (Weigert-Hart's elastic stain, x 840).

In such cases, the internal elastic lamina edge of the intima, i.e. the edge with distinguished deposited lipids is the one that is mostly changed, with a scalloped form (fig. 6). Meanwhile, the lower edge of the elastic plaque, adjacent to the middle coat, registers no visible changes almost to full lysis of internal elastic lamina, which serves as confirmation of the secondary character of dystrophic changes in the elastic membrane in response to lipid deposition. In more advanced stages of internal elastic lamina decomposition, the latter is missing at a considerable distance or exists as fragments and lumps [3].

The intensive destruction of elastic structures of internal artery tissue is a reflection of general metabolic changes that take place in the vascular wall, including tissue hypoxia, release of hydrolytic lysosomal enzymes, tissue acidosis, deposition of immunoglobulin with formation of an autoimmune complex, intima imbibition with plasma proteins, penetration of elastase and blood plasma.

In the early stages of atherosclerosis, one may observe the formation of cholesterol crystals in foam cells and in the extracellular space. Although a relatively inert part of cholesterol, however, cholesterol crystals can cause mechanical tissue damage, exacerbating the atherosclerotic process. Unlike cholesterol esters, its crystals are not absorbed by the cell, so, even in regression, they stay in the arterial wall.

The formation of cholesterol crystals between atheromatous masses is related to the decay of foam cells and release of their content into the intercellular space, including membrane-lacking lipid vacuole, consisting of cholesterol esters (fig. 7).

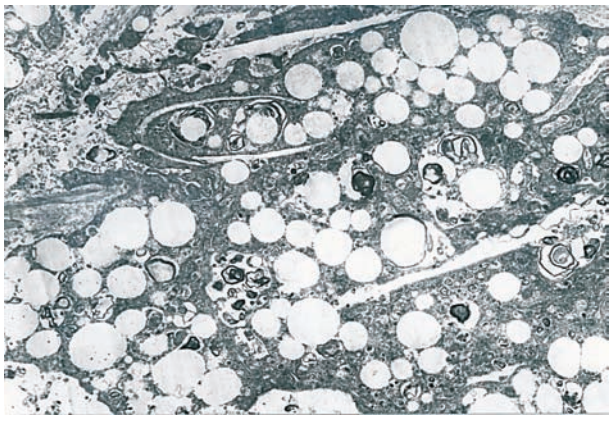

Fig. 7. Atherosclerotic plaque of basilar artery. Formation of cholesterol crystals in the site of decayed foam cells of smooth muscle origin, with lipid vacuole in cytoplasm (x 60000).

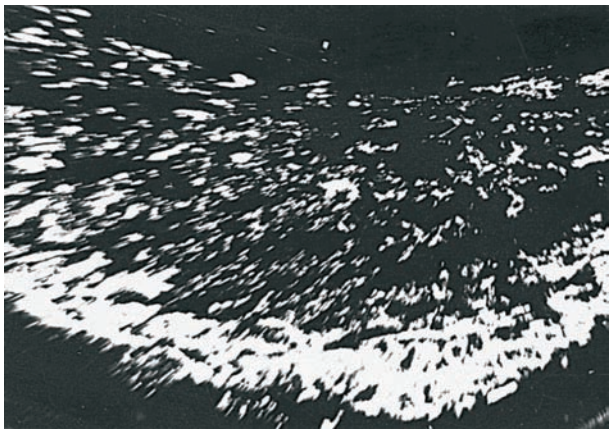

Fig. 8. Polarizing microscopy shows that the cholesterol crystals are deposited along the internal elastic lamina of the basilar artery (x 460).

The accumulation of cholesterol crystals is mainly concentrated along the internal elastic lamina (Fig. 8), which can also cause elastic damage and lysis.

On destruction of the elastic membrane and penetration of lipids into the middle tissue, intima foam cells can be formed in situ, i.e. in tunica intima and in the sites surrounding it. Meanwhile, smooth muscle cells of the subintimal layer penetrate intima through affected elastic areas, contributing to further disintegration of internal elastic lamina. Smooth muscle cells participate in the formation of connective tissue components, including collagen and elastic fibers (fig. 9-12). On lipid penetration into tunica media, in the sector under

adventitia, one observes an accumulation of macrophages and lymphocytes that create infiltrates. The infiltration of macrophages through the membrane in lipid deposition sites is also possible.

Fig. 9. Lipids penetration in the media through internal elastic lamina defect (Oil Red staining, x 460).

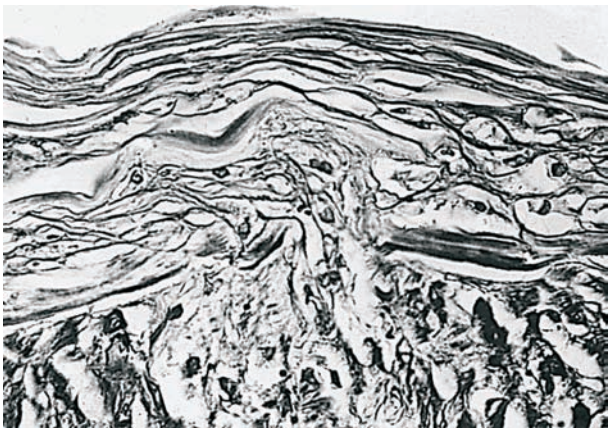

Fig. 10. Migration of smooth muscle cells through internal elastic lamina defect (Weigert's stain method, x 460)

Fig. 11. Migration of mononuclear cells through internal elastic lamina defects in the atherosclerotic plaque, with formation of infiltrates in the deep layers of the plaque (Weigert's stain method, x 460).

Alongside with the decay of vascular wall fibrous structures, a formation of new structures occurs. Yet, the elastic fiber-forming processes are manifested much weaker than the appearance of collagen fibers. There are many reasons for this phenomenon. Essentially, all factors that lead to lipid deposition in intima and metabolic disorders of the vascular wall are reasons that contribute to the development of connective tissue and atherosclerotic fibrous plaque formation.

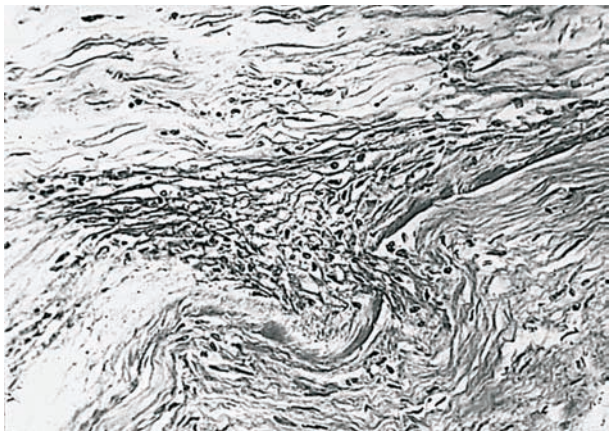

Fig. 12. Formation of precollagen fibers that cover internal elastic lamina defects (Weigert–Hart's stain method, x 460).

To some extent, the newly-formed elastic fibers cover the fiber defects, especially over the damaged internal elastic lamina. Thin elastic fibers are located in the muscular elastic layer between acid glycosaminoglycans. Particularly noteworthy is the genesis of new elastic fibers in the cerebral basilar artery, where they are formed even before complete lysis of the internal elastic lamina. The newly-formed elastic fibers, in turn, are subject to dystrophic changes. They stick together, are intensely stained with fuchsin, mix; in some places, they are subject to lysis and gradually dissolve into the surrounding mass of lipids and proteins (fig. 13).

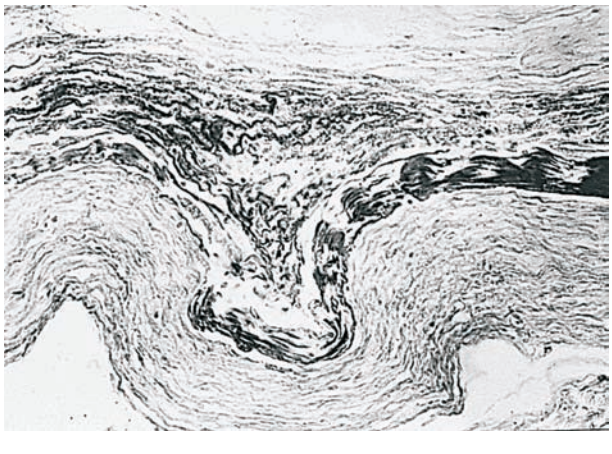

Fig. 13. Homogenization, lysis and fragmentation of newly-formed fibers in sites of internal elastic lamina rupture, which partially cover the lamina defect in the subendothelial layer of foam cells (Weigert–Hart's stain method, x 840).

Together with lipids deposition on elastic fibers, they also accumulate in intima argyrophilic structures and on collagen fibers on the layer connecting the plaques. As to their appearance, lipid deposits on argyrophilic structures and collagen fibers are somewhat different from lipid deposits on elastic

membranes. Changes taking place in argyrophilic structures are quite difficult to follow, as they are very closely attached to elastic fibers, weaving them around. This is the reason why, for example, in the cerebral basilar artery, in sites of internal elastic lamina lysis, one can observe the formation of only a few elastic fibers covering the lysate lamina defect, while the formation of argyrophilic fibers takes place throughout the thickness of atherosclerotic plaques (Fig. 14).

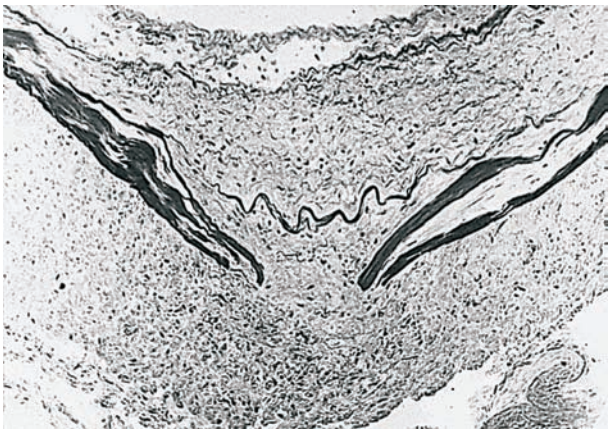

Fig. 14. Tumefaction, fragmentation, lysis and disintegration of internal elastic lamina; partial defect "covering" with newly-formed elastic fibers. Foam cells accumulation in the subendothelial layer (Weigert–Hart's stain method, x 840).

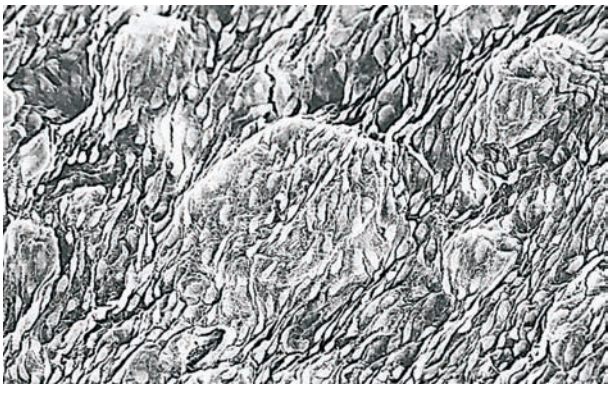

Fig. 15. Scanning electron microscopy. Swollen endothelium focus above the site of lipid deposition in intima (x 3200).

Concurrently, an increased content of collagen fibers is observed in such areas. The argyrophilic fibers newly formed in atherosclerotic plaques lose their characteristic tortuosity, stick together, as well as to elastic fibers, and impregnate intensely with silver.

Increased production of collagen fibers results in a thickening of the inner layer. Intima and subintimal layer, even in normal conditions, especially in the elderly, are constantly at anoxia limit. In anoxia, elements of connective tissue are intensively formed, leading to thickening and deformation of artery walls.

On analysis of basilar artery surface with the scanning electron microscope, above the lipid spots and plaques, one observes a characteristic feature of atherogenesis – the endothelial monolayer change. The endothelium, on the surface of edema and lipid deposition in focus in intima, takes the form of a cobblestone pavement, with deposits of erythrocytes in the folds of prominent cells (fig. 15).

Two types of endothelial cells can be distinguished above the emerging atherosclerotic plaques [4]. The first type consists of functionally activated endothelial cells; in their cytoplasm, one observes an increase in the number of hypertrophic mitochondria, concentrated at nucleus poles; expansion of rough (granular) endoplasmic reticulum cisternae; a large number of ribosomes and unlimited endocytic vesicles; Weibel-Palade corpuscles are prominent. The second type consists of endothelial cells with irreversible dystrophic and degenerative changes, with a background of formation of large lipid vacuoles in the rough endoplasmic reticulum.

Along with destructive processes in the basic elements of the vascular wall - destruction of elastic, collagen fibers and cellular elements: endothelium, SMCs, monocytes, macrophages, fibroblasts, there also take place compensation, adaptive-regenerative processes, accompanied by formation of new blood vessels - neoangiogenesis (neovascularization). We studied this process by means of special research methods.

The intima of newly-formed vessels, associated with atherosclerotic plaques, was firstly studied in 1876 by Koester. In atherosclerotic plaques, angiogenesis allows for the formation of new microvessels, in order to maintain the required level of oxygen and nutrients in the vascular wall.

CD105 is a homodimeric membrane glycoprotein, complete, consisting of 90-95 kDa subunits, bisulphite-bound, and is part of the transforming growth factor beta receptor TGF-β. CD105 is manifested in angiogenic endothelial cells [5]. Thus, CD105 is a sensitive marker for identifying newly-formed blood vessels [6]. Concurrently, CD105 is a more specific and sensitive marker for the evaluation of newly- formed blood vessels in atherosclerotic plaques than CD31 or TGF-β1 [7, 8].

Determining the level of circulating soluble CD-105 - sensitive antigens - allows us to determine exactly the presence of unstable plaques or their disruptions [9].

Neovascularization occurs in sites of atherosclerotic lesions undergoing constant change, reconstruction and prone to rupture. Some studies show that the formation of new blood vessels contributes to the growth of atherosclerotic lesions and is a key factor leading to destabilization and disintegration of the plaque.

Some of the newly-formed blood vessels are immature, similar to those observed in the neovascularization of solid tumors and, therefore, can lead to tearing and bleeding in the plaque site, and subsequently - to its instability.

Mast cells, macrophages and T-lymphocytes, which interact together, usually form inflammatory cell infiltrations of atherosclerotic plaques and intercellular substance [10].

Material and methods

Using immunohistochemical methods, we studied a number of different brain vessels in 34 patients with cerebrovascular disease (ischemic and hemorrhagic stroke). The patients included representatives of all age groups, but one

Table 1

Immunohistochemical methods used in the study

Antibody	Source	Clone	Dilution	Detection system	Antigen retrieval	Primary antibody incubation
Alpha Smooth Muscle Actin	Novocastra, Newcastle upon Tyne, UK	Human Alpha Smooth Muscle Actin, ɑsm-1 clone	RTU	NovoLink Max Polymer Detection System (Novocastra Newcastle upon Tyne, UK)	Microwaves, 5 minutes, pH 6	30 minutes, room temperature
CD68	Dako Glostrup Denmark	Monoclonal mouse anti-human, QBEnd 10	1:25	NovoLink Max Polymer Detection System	Microwaves, 5 minutes, pH 6	30 minutes, room temperature
Endoglin	Dako Glostrup Denmark	Monoclonal mouse anti- human, clone SN6h	1: 10	NovoLink Max Polymer Detection System	Proteinase K, 10 minutes	30 minutes, room temperature
CD34	Dako Glostrup Denmark	Monoclonal mouse anti-human, 1A4	RTU	NovoLink Max Polymer Detection System	Microwaves, 30 minutes, pH 6	30 minutes, room temperature
Mast cell tryptase	NeoMarkers, Fremont, CA	Mouse Monoclonal Antibody, AA1clone)	RTU	NovoLink Max Polymer Detection System	Microwaves, 30 minutes, pH 6	30 minutes, room temperature

could notice that the extent of damage increases past the age of 40. The age range was between 44 and 83 years (mean age – 62, 8 years). Women accounted for 41, 2%, men – 58, 8%. During autopsy, fragments of cerebral arteries were taken for study.

Vascular fragments were processed by standard method (fixing in 10% buffered formalin solution, embedding in paraffin blocks; 4-5 micrometers thick sections were obtained). The determination of the type and stage of plaques was based on AHA (American Heart Association, 1995) morphological classification, considering the macroscopic and histological image of sections stained with hematoxylin-eosin, as well as the histochemical techniques - silver and orcein impregnation.

Additional sections of paraffin blocks were processed immunohistochemically; they were deparaffinized, hydrated, subject to reaction for detection of antigen in the PT Link module (Dako Cytomation Denmark). The next step was the primary antibody incubation, using NovoLink Max Polymer Detection System, and, to visualize the final reaction, we used 3, 3'-diaminobenzidine dihydrochloride as brown chromogen.

CD105 interpretation: we quantified vascular structures with lumen, positive for CD105, brown-colored at cytoplasmic level in endothelial cells. The microvascular density was determined using the hot spot method [11, 12].

Concomitantly, we also considered the positive signals for CD105-positive endothelial cells, capillaries and plexi of CD105 positive endothelial cells, as well as in periplaque site and in the plaque itself.

The histopathological analysis revealed the existence of three major (conditional) types of atherosclerotic lesions in two study groups: intermediate injury (II), fibrous (formed) plaque (FP), calcified (and/or complicated) fibrous plaque (CFP) in all the studied vessels, which, in turn, were stained with hematoxylin-eosin, orcein and were impregnated with silver.

Score of antibody expression in arterial vessel walls and atherosclerotic plaques

Score	Immunolabeling	Positive cells (%)	Intensity
0	-	<1%	-
1	+	1-25%	Low
2	++	26-50%	Moderate
3	+++	>50%	High

Results

Table 3

Biochemical laboratory data of the patients included in the study

Age	63,4 (average)
Gender	21/13 (m/f)
Glycemia	4,74 (mmol/l)
Total cholesterol	6,90 (mmol/l)
Triglycerides	0,86 (mmol/l)
High-density lipoproteins	1,003 (mmol/l)
Low-density lipoproteins	1,99 (mmol/l)
Leukocytes	8,42 (×109/l)
Lymphocytes	24, 5 (%)
Monocytes	7, 57 (%)
Prothrombin	73, 6 (%)
Fibrinogen	3,3 (g/l)
Erythrocyte sedimentation rate	17,5 (mm/h)

These images, studied in optical microscopy of the basilar

Table 2

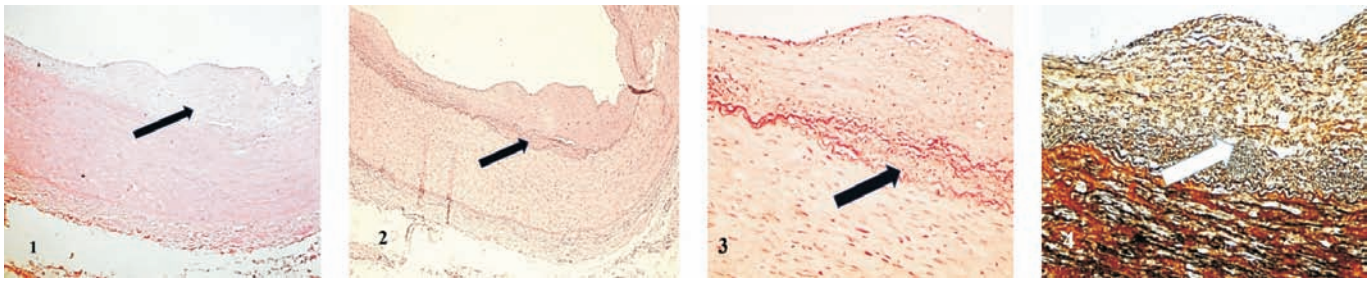

Fig. 16. Fibrous atherosclerotic plaque of basilar artery.
1. The fibrous plaque (H-E x 20); 2, 3. The disintegration of elastic lamina (Orcein, x 20, x 40);
4. Elastic and reticular fibers (Silver Impregnation, x 40).

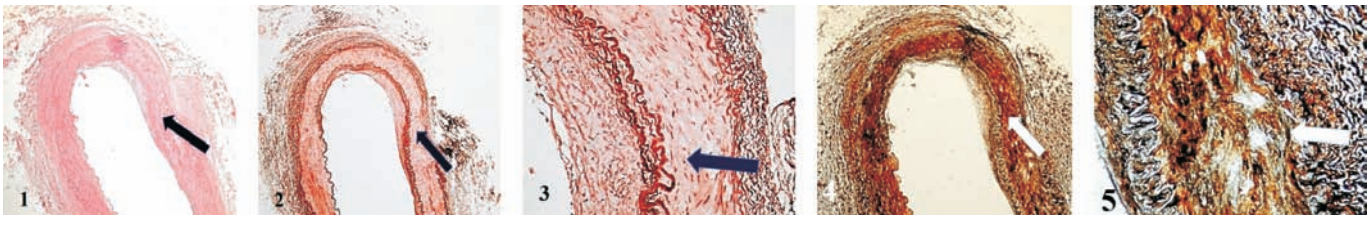

Fig. 17. Fibrous atherosclerotic plaque of basilar artery.
1. The fibrous plaque (H-E x 10); 2, 3. The disintegration of elastic lamina (Orcein, x 10, x 20);
4, 5. Elastic and reticular fibers (Silver Impregnation, x 10, x 40).

Immunohistochemical methods demonstrate the existence of the anti-MCT, CD68 and CD105 (endoglin) differential expression in different types of atherosclerotic lesions and various types of vessels in combination with histotopographic distribution.

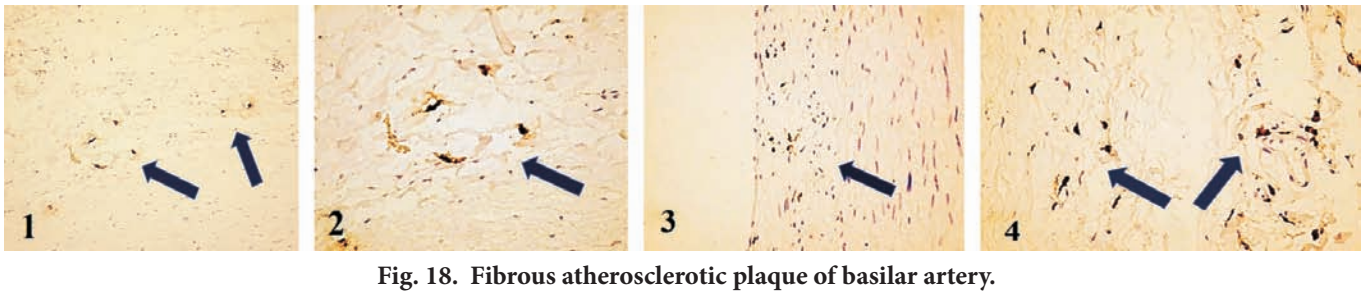

Fig. 18. Fibrous atherosclerotic plaque of basilar artery.
1, 2. The fibrous plaque (anti-MCT, x 20, x 40); 3, 4. The fibrous plaque (anti-CD 68, x 20, x 40).

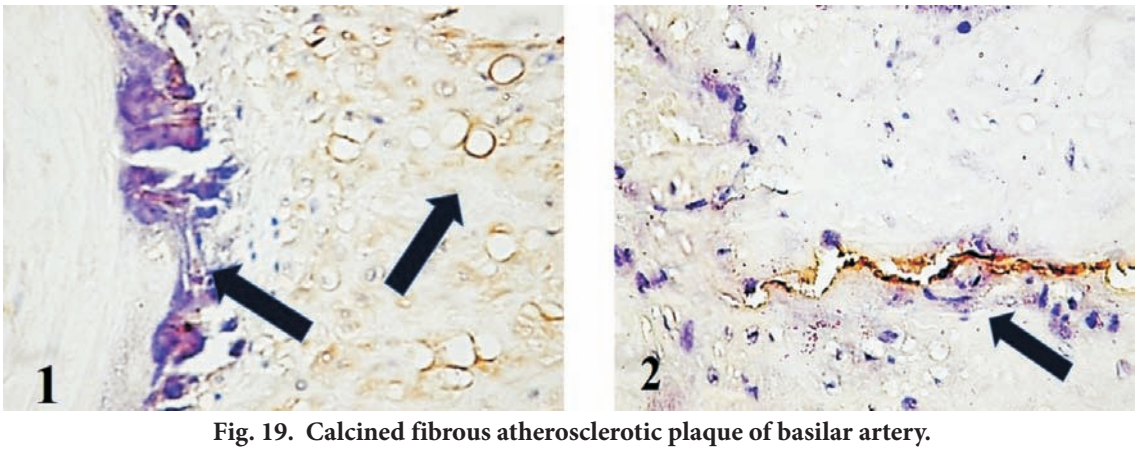

Fig. 19. Calcined fibrous atherosclerotic plaque of basilar artery.
1. 2. The calcined fibrous plaque and microvessels (anti-CD 105, x 40).

artery and applying histochemical staining methods, reflect structural changes of the vascular wall components, characteristic to fibrous plaque stages. The most representative are the disruption of the endothelial layer, with its significant thickening, atherogenic masses storage at the subendothelial level, endotheliocytes hyperplasia (Fig. 16.1 and 17.1, hematoxylin-eosin-stained x10, x20). On applying orcein staining with x20, x40 magnification on these sections, in Fig. 16.2, 16.3, 17.2 and 17.3 one clearly notices the changes at the level of lamina propria, accompanied by its thickening, deformation and splitting, something that is not characteristic for intact blood vessels. The basal membrane, with endotheliocytes on it, is split at the level of fibrous plaque and one also observes here atheromatous masses, fibrous tissue, thus explaining the specific deformation of fibrous plaques in atherosclerotic damage. Silver impregnation with x10 and x40 magnification in Fig. 16.4, 17.4 and 17.5 allows highlighting elastic and reticular fibers at the plaque level, with complex disruption of extracellular matrix. The observed optically blank areas are areas of lipids accumulation, surrounded by thick and deformed fibers. In some complicated plaques, they have a chaotic layout and can be combined with calcium deposits, hemorrhagic imbibition and inflammatory cellular components in unstable plaques, which, in turn, can be complicated by rupture, with clinical manifestations of fatal acute hemorrhagic strokes.

On analysis of immunohistochemical expression of cellular components involved differently and dependent on the evolution stage of atherosclerotic plaques, a diverse participation of inflammatory cells in different compartments of the vessel wall is defined. In Fig. 18.1 and 18.2 anti MCT x20, x40 magnification, one observes mast cells positively brown stained with the specific marker for these anti-MCT (tryptase mast cells). The distribution of mast cells is varied and proportional to the evolution stage of atherogenic process in outbreaks of inflammatory reactions. More frequently, mast cells are located in the subendothelial site and around the inflammatory process, as well as in the plaque, if the inflammatory process extends therein.

On the other hand, in various evolution stages, along with mast cells, one can also observe macrophages, which are highlighted with the marker selectable for them - CD68 (macrophage cells are positively brown stained) in Fig. 18.3 and Fig. 18.4, x20, x40 magnification. Like mast cells, macrophages also have a varied distribution in the plaque, depending on the immunoreactive condition and the evolution stage of the atherogenic process. The number of mast cells and macrophages is varied, but some features were observed in the laws of their expression - mast cell growth is characteristic for acute inflammatory reactions, while macrophages growth occurs at the decrease of acute inflammation stages. Their strictly defined functions explain their important role in inflammatory processes in general and in the development of atherosclerotic plaques, in particular.

Another important pathogenetic link is atherosclerotic angiogenesis, shown in Fig. 19. The atherosclerotic angiogenesis, like the tumoral one, has some similar evolutionary features. The specific marker CD105 (endoglin) highlights in brown the vessel endothelium newly formed around atherosclerotic plaques and, in some cases, inside them. The expression of positively stained endothelium and the newly-formed capillaries is varied and dependent on many factors. In Fig 19.1 and 19.2 x40 magnification, one observes a complicated atherosclerotic plaque, with calcinosis focus, with multiple newly-formed vessels, which can form clumps of endothelial cells, plexuses and capillaries with prominent lumens.

Conclusions

The information obtained confirms the evolution theories and the pathogenic mechanisms of atherogenesis. Despite these assertions, at present, there are a lot of unresolved scientific and clinical issues.

For comparison, one may bring as example the angiogenesis of tumoral processes, in which mast cells and macrophages, similar as in the processes of formation of atherosclerotic plaques, are involved in neovascularization (only when discussing mechanisms, general functions and pathophysiological links).

Anti-MCT and CD68 are selective markers for mast cells, macrophages, which are important components of immune processes, in the initiation, proliferation and differentiation of cells in atherosclerotic lesions. Along with T-lymphocytes and macrophages, other immune effector cells are involved in atherosclerotic lesions, while lymphocytes and macrophages prevail over mast cells, which play an important role in the development of atherosclerotic plaque in different vessels. This fact can be explained by the generation of large quantities of proteases, including those produced by macrophages, with their accumulation in the necrotic nucleus site of the plate.

The factors produced by mast cells and macrophages can cause the destruction of intercellular matrix and a further change of low-density lipoproteins. Most examined vessels were positively stained by anti-MCT and CD68 at the level of endothelium, atherosclerotic plaque, in the site of the media and the adventitia, as well as vasa vasorum.

Endothelial cells, mast cells, macrophages and lymphocytes are, obviously, effector cells involved in atherogenesis, with development of atherosclerotic plaques in patients with atherosclerosis of cerebral vessels. Mast cells regulate the conduct of smooth muscle cells (SMCs), probably through their secreted mediators. Collagen fibers produced by smooth muscle cells can prevent rupture of atherosclerotic plaques. Yet, the chymase inhibits mast cell proliferation and collagen synthesis by SMC, thus reducing the stability of the plaque.

The action of proinflammatory cytokines, such as TNF-α, induces the expression of SMC protease. TNF-α-positive mast cells, MMP-cysteine, cathepsin-positive SMC proteases, together with macrophages, suggest a regulatory role in the expression of cellular mediators, mast cell proteases in SMC activation in sites of atherosclerotic plaque rupture. The position of mast cells and macrophages in the vascular wall, especially perivascularly and at intima level assumes an important role in the pathogenesis of atherosclerosis and is probably the main cause of acute cardiovascular diseases

(especially myocardial infarction and stroke).

The role of angiogenesis in the development of atherosclerosis is a complex one and depends on the stage of the pathologic process. Microvessel development in atheromatous plaques is the outcome of neovascularization; these newly-formed capillaries are fragile and prone to rupture with bleeding. Fibrin deposition in plaque, hemosiderin formation and onset of immune inflammation constitute bleeding evidence in atherosclerotic lesions. The role of angiogenesis in atherosclerotic plaque destabilization and destruction remains an open question, but some recent judgments about the primary causes of plaque instability can lead to a promising new interpretation of atherogenesis in general.

The laws of atherosclerotic plaque development (stability and instability) depend largely on the angiogenesis of the atherosclerotic process. Our results show that the comparative immunohistochemical method using vascular markers demonstrates significant pathogenetic aspects in atherosclerotic plaque formation. Macrophages, mast cells and other immune cells play an important role in the development of atherosclerotic plaques and, not the least, in the process of angiogenesis. The question arises: can the angiogenesis inhibition be a therapeutic target in atherosclerosis or how can it be used in metabolic syndrome?

Available data suggest that anti-angiogenic therapy may have a potential impact on the development of neointima in atherosclerotic lesions, and the side effects and harmful factors are likely to inhibit the function of the endothelium and its regeneration. These statements are supported by scientific evidence of a large number of laboratories showing that VEGF has a protective effect on the endothelium of arteries. Recent clinical studies of VEGF inhibitor antibodies, at the administration of bevacizumab, avastin in malignant tumors, indicate that up to 5% of patients treated with avastin may be at increased risk of thromboembolic events, including acute strokes, myocardial infarction and phlebothrombosis. These data allow us to assume that endogenous VEGF may play a certain atheroprotective role in vascularization. The multiple significant biological functions of VEGF and the integrity of vascular endothelium functions are solid arguments that currently limit any anti-angiogenic approach for the treatment of cardiovascular diseases.

In conclusion, we mention that there is a close relationship between plaque morphology and clinical manifestations in patients with atherosclerosis of cerebral arteries. Positive remodeling and a larger plaque site can be found in the mobile plaque, while negative remodeling and smaller plaque site can be found in the stable plaque. The degree of CD105 solubility is linked to the characteristics of the plaque and can serve as a predictor for the soluble plaque. Large sample studies are needed to prove whether soluble CD105 can predict atherosclerotic plaque rupture.

CD105 is a valuable marker of angiogenesis of atherosclerotic plaques, intimal arteries and adventitial vessels, an indicator of the difference degree in the pathological development of atherosclerosis, all these factors that may be of great importance to the introduction of modern methods of research, diagnosis, treatment and prognosis of these diseases.

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Experience of use of endorectal high dose rate brachytherapy in neoadjuvant treatment of the locally advanced rectal cancer

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Abstract

Background: The aim of the present study is to assess the response rate and toxicity profile in patients with locally advanced rectal cancer using high dose rate endorectal brachytherapy (HDR-EBT) as a start component of the neoadjuvant treatment.

Material and methods: 28 patients with T3-4N0-2M0 rectal adenocarcinoma were included in the study. A novel approach using HDR-EBT is given in 4 fractions (4 Gy per fraction, 2 times a week) in combination with external beam radiotherapy (EBRT) 30,6 Gy (1,8 Gy per fraction). All patients received neoadjuvant chemotherapy during the course of irradiation consisting of Capecitabine 825 mg/m2 per os daily.

Results: The majority of patients were males (n=16; 57.1%), 12 (42.9%) – were females, their mean age was 60,6 years. All patients had a decrease in tumor size from average of 4,88 cm to 3,14 cm longitudinally. 21 of 28 patients (75%) had sphincter preserving surgery. 17 of 28 patients (60.7%) had a pathologic complete response of their primary tumors. Radiation therapy was well-tolerated. Acute GI and GU toxicity was limited to ≤ Grade 2 for all patients. Local recurrence in the observation group within 2 years was 3.6%.

Conclusions: The use of HDR-EBT as a start component of the neoadjuvant locally advanced rectal cancer treatment is an acceptable modality with high pathological response rate as well as an acceptable toxicity profile.

Key words: locally advanced rectal cancer, high dose rate, endorectal brachiotherapy.

Introduction

Neoadjuvant chemoradiotherapy with further rectal cancer surgery at the present day is a standard in the locally advanced rectal cancer (RC) treatment [1]. It is known that use of high dose rate endorectal brachytherapy (HDR-EBT) at the preoperative stage in the treatment of RC especially the low-localization tumors, leads to an improvement of a local control and possibility of the sphincter-saving procedure [2]. In spite of the resounding success of use of an intracavitary irradiation in complex curative or individual palliative therapy of RC, the problem of use of a contact gamma-therapy at this nosology is remote from a final solution. In particular, bright-line rules at the level of a cumulative doze (CD) from the present component of the radiotherapy, fractionating regimes and, in particular, an order of combination of HDR-EBT with other methods of the antineoplastic impact are absent.

The aim of the present study is to assess the response rate and toxicity profile in patients with locally advanced RC using HDR-EBT as a start component of the neoadjuvant treatment.

Material and methods

28 patients with the locally advanced primary unresectable RC of stage T3-4N0-2M0 were examined and they underwent a treatment due to the submitted regimen in 2011-2013 in Kherson Regional Oncological Dispensary.

Patients underwent HDR-EBT given in 4 fractions (4 Gy per fraction, 2 times a week) in combination with external beam radiotherapy (EBRT) 30,6 Gy (1,8 Gy per fraction). All patients on the first day of the radiotherapy received HDR-EBT session, in further HDR-EBT was conducted 2 times per week (Monday, Thursday), and EBRT was placed on days free of HDR-EBT.

EBRT was conducted with the use of device "Teragam K-01" (source activity 60Co 177 TBq, 1,17-1,33 MeV) per pelvis region. Lower limit of fields was situated 2 cm lower of an anal edge, upper limit - at the level L5-S1, lateral – 1 cm laterally of a bone pelvic ring. For the performance of 3D-planning on the system «PlanW 2000», axial plane CT series were used, that eventually led to the formation of the opposite fields 16–16 × 18–18 cm depending on the constitutional particulars of a patient.

HDR-EBT was conducted with the use of device «MultiSource» (source activity 60Co 70 TBq). Before the conduction of HDR-EBT a tumor size, a depth of the rectal wall invasion, a tumor prevalence were considered with the relation to the anal canal. Type and sizes of an applicator were chosen depending on a radiation area and anatomy of a patient. Planning was performed on the basis of MRI in a planning system «HDRplus 2.6».

Diagnosis of the underlying disease of all patients was morphologically verified. All tumors were an adenocarcinoma G2. All patients underwent radiotherapy with a Capecitabine radiosensitization with a daily doze of 825 mg/m2 per os.

There were men 57.1% (n=16) and women – 42.9% (n=12) under investigation. Age of patients varied within the range of 39-77 years (on average 60.6). Tumors of the rectal lower ampulla were present in 12 (42.9%) and the rectal middle ampule – in 16 (57.1%) cases. Pain was present in 20 (71.4%) of patients before treatment given. Chronic rectal bleeding was present in 13 (46,4%) of patients. 9 (32,1%) of patients suffered from a constipation at the beginning of the treatment. Distance between an anus and a distal pole of a tumor before therapy amounted to 2-9 cm (on average 5,55 cm). Extension of a primary tumor along the length of a rectum amounted from 2 to 10 cm (on average 4,88 cm).

Results and discussion

Total pain relief after the treatment given was reached in 12 (42.9%), partial pain relief – in 8 (28.6%) of patients. Chronic rectal bleeding was negated in 92.9% of cases. Constipation disappeared for the moment of the treatment termination in all cases. Distance between an anus and a distal pole of a tumor after therapy increased on average to 1,35 cm, extension of a primary tumor diminished to 3,14 cm (on 35.6%).

Some studies showed that combined radiotherapy is more effective in rectal tumor downstaging than EBRT alone and achieves a significant improvement in sphincter-saving procedure up to 76% in T2-T3 RC [3]. RC downstaging into a resectable stage was evaluated during a control examination in 4-6 weeks after completion of a radiotherapy (MRI, edoscopy) in our study, and amounted to 96.4% of cases (27 patients). 6 (21.4%) of patients were not candidates for sphincter-saving procedures. The number of the sphincter-saving procedures amounted to 21 (75%). Surgery was not performed in 1 (3.6%) patient. Reason of rejection of a surgery treatment was unresectable tumor. In the post-surgery period 1 case of sigmoidoproctostomy deficiency and 1 postoperative urinary bladder atony were noticed that amounted to 3.6% (total number of the postoperative complications didn't exceed 7.2%, which is comparable with statistical number of the postoperative complications in Ukraine and in the world).

It is known that HDR-EBT has advantages in the tumor destruction, but it also has disadvantages which are associated with the risk of complications [4]. During the whole course of a radiotherapy any undesirable effects were absent for 6 (21.4%) patients. One undesirable effect was noticed in 19 (67.9%) patients, simultaneously two — in 3 (10.7%) patients. Three and more undesirable effects were not registered. Absence of the toxic reactions of grade III and grade IV was detected. Intensity of the general complications in all cases corresponded to grade I according to the CTCAE 4.0.

El Sayed (2014) et al. and Hacker-Prietz (2015) reported cases of grade III proctitis post- HDR-EBT [5, 6]. In our study radiation proctitis of grade I and grade II developed with the same frequency, 5 (17.9%) cases. The cases of grade III proctitis were not registered.

There was only grade I radiation cystitis noticed in 3 (10.7%) patients. Grade I radiation epidermis was noticed in 1 (3.6%) patients in our study. El Sayed (2014) et al. reported no patients developed grade III cystitis, but grade III dermatitis in that study was seen in 12% of patients [5].

Anaemia developed in 1 (3.6%) patient, leucopenia – in 6 (21.4%) patients. Granulocytopenia, lymphopenia and thrombocytopenia were not detected. General type reaction (weakness, ailment, inappetency, low-grade fever) was noticed in 3 (10.7%) patients. Vomiting was not observed, sicchasia was registered in 1 (3.6%) patient, in this case this toxicity didn't exceed grade I.

Complete pathological response was noticed in 17 (60.7%) patients, pathological partial response was registered in 10 (35.7%), absence of pathologic changes was noticed in 1 (3.6%) case. Our results are hopeful because not numerous research of HDR-EBT effectiveness for RC treatment showed complete pathological response up to 33% [6] and 47% [5].

Local recurrence developed within 2 first years of monitoring in 1 (3.6%) patient. No case of a distant metastasis during the whole period of monitoring was detected. 2 (7.1%) fatal cases were registered. The cause of death of both died persons was a pathology unrelated to cancer (in one case was an injury, in the second one – a heart failure).

Conclusions

The use of HDR-EBT as a start component of the neoadjuvant locally advanced rectal cancer treatment is an acceptable modality with high pathological response rate as well as an acceptable toxicity profile.

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S100 protein expression in pituitary adenomas

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Abstract

Background: The predicting of pituitary tumor behavior remains one of the most incurable medical problems. One of the causes is poor correlation between the morphology of pituitary tumors and their clinical aggressiveness.

Material and methods: 96 cases included in our study have been microscopically investigated on specimens colored with eosin haematoxylin by three experienced pathologists according to WHO recommendations. Out of these ten cases were represented by normal pituitary tissue to make it possible to compare the typical pituitary morphology with the microscopic appearance of various types of pituitary adenomas.

Results: In both the normal pituitary gland and in pituitary adenomas, endothelial cells presenting a nuclear expression for S100 were occasionally observed. The percentage of positive cases for protein S100 was 66.12% from the total number of cases. Amongst these, 39.02% presented a compact growth pattern, 39.04% were of papillary type, 9.75% presented a trabecular growth pattern, 4.87% spindle-shaped and 7.31% were of alveolar type. Papillary type pituitary adenomas registered the highest intensity of expression for protein S100 in tumor cells. The acidophilic cells were present in a percentage of 34.2% of cases. Pituitary adenomas with basophilic cells represented a percentage of 26.8% of positive cases for protein S100, and, for 39% of cases the chromofobe component was present forming pure chromofobe pituitary adenomas or mixed chromofobe-acidophilic/basophilic pituitary adenomas.

Conclusions: Protein S100 expressions in tumor cells is implicated in the pathogenesis of the growth hormone and prolactin secreting pituitary adenomas, the mechanisms of activation being nowadays incompletely studied. Through analogy with the observations obtained in other tumor types, it is possible that S100 pituitary adenomas to represent a group of pituitary adenomas with an aggressive behavior and a high capacity of invasion and recurrence, aspects that represent an unfavorable prognostic factor.

Key words: S100 protein, human pituitary adenoma.

Introduction

Predicting of pituitary tumor behavior remains one of the most incurable medical problems. One of the causes is poor correlation between the morphology of pituitary tumors and their clinical aggressiveness. Pituitary tumors have been strongly scrutinized for their immunohistochemical expression of a wide range of proteins, growth factor, cytokines and various gene products in hope to find prognostic markers. The aims of the present work were to study the presence and distribution of S100 protein-immunoreactive cells to assess their response in cases of various types of pituitary adenomas. Protein S100 was originally isolated in the central nervous system and has been localized in folliculo-stellate sells of the anterior lobe of the pituitary gland.

Material and methods

The research was carried out at the Department of Morphopathology of Nicolae Testemitsanu State University of Medicine and Pharmacy in 2012-2015 period. The research protocol has got the Research Ethics Committee approval (protocol No 52 of 08.06.2015)

96 cases included in our study have been microscopically investigated on specimens colored with eosin haematoxylin by three experienced pathologists according to WHO recommendations. Out of these ten cases were represented by normal pituitary tissue to make it possible to compare the typical pituitary morphology with the microscopic appearance of various types of pituitary adenomas.

The studied samples were taken from the postoperative pieces (surgically removed pituitary adenomas), which were set in a 4% formalin solution buffered at pH 7.2 for 48-72 hours and placed into paraffin, using the common histological technique Thermo Shandon standardized and automated system (Thermo Fisher Scientific Inc., Waltham, MA, USA). The microscopy was performed using Nikon Eclipse E600 microscope (Nikon Corporation, Tokyo, Japan), the images being taken with Coolpix 950 digital camera in JPEG format. The data are presented as absolute and relative expressions (%).

Results

Protein S100 in pituitary adenomas was also studied by using the immunohistochemical method on the normal pituitary gland and in pituitary adenomas. Protein S100 proved to have a nuclear and cytoplasmic pattern, being positive in the stellate follicular cells but also in the endocrine cells of the normal pituitary gland (fig. 1) and the adenomatous ones.

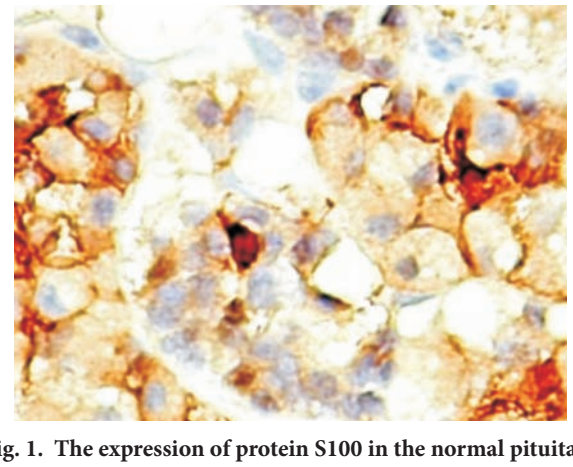

Fig. 1. The expression of protein S100 in the normal pituitary gland. Note the intense, nuclear and cytoplasmic expression in the stelate follicular cells and the moderate cytoplasm-restricted expression in the endocrine cells with an acidophilic pattern on hematoxilin-eosine and a low intensity in chromofobe cells.

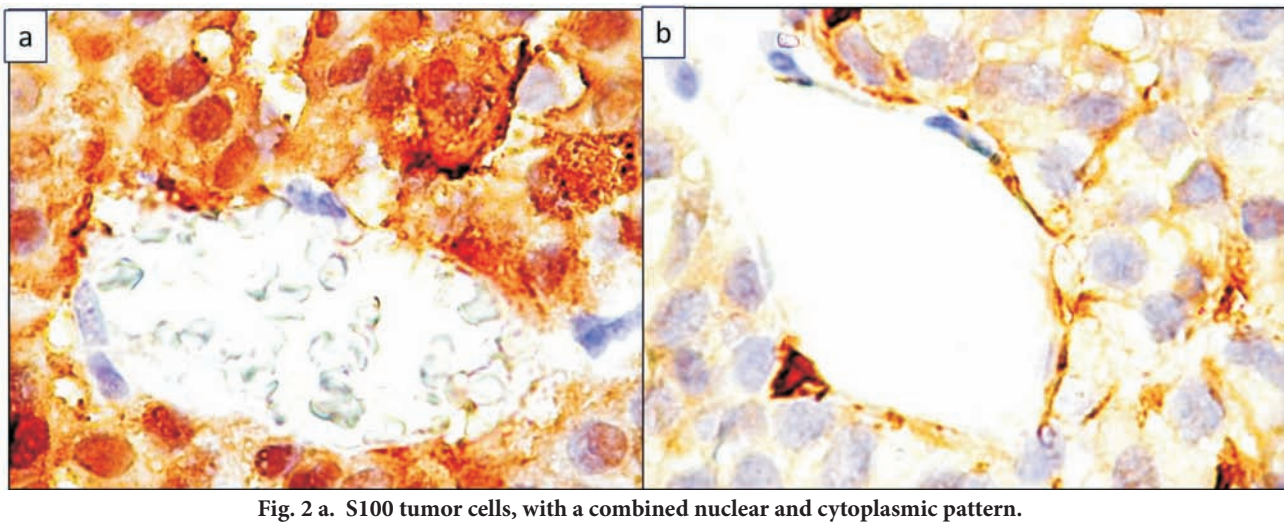

Fig. 2 a. S100 tumor cells, with a combined nuclear and cytoplasmic pattern.
Fig. 2 b. S100 tumor cells, exhibiting a cytoplasmic pattern.

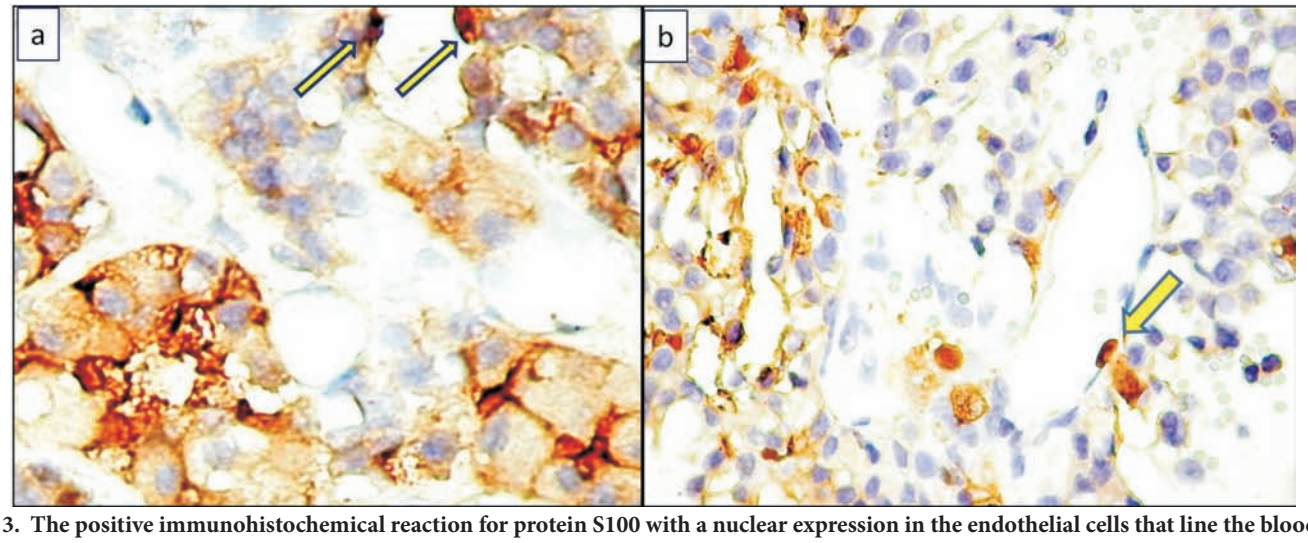

Fig. 3. The positive immunohistochemical reaction for protein S100 with a nuclear expression in the endothelial cells that line the blood capillaries from the normal pituitary gland (a, arrow) and from the vessels belonging to pituitary adenomas (b, arrow).

The combined nuclear/cytoplasmic pattern was observed in tumor cells in only 19.5% of S100 positive cases (fig. 2 a), the rest of the cases presenting a cytoplasm-restricted expression (fig. 2 b).

In both the normal pituitary gland and in pituitary adenomas, endothelial cells presenting a nuclear expression for S100 were occasionally observed (fig. 3 a, b).

The percentage of positive cases for protein S100 was 66.12% from the total number of cases. Amongst these, 39.02% presented a compact growth pattern, 39.04% were of papillary type, 9.75% presented a trabecular growth pattern, 4.87% spindle-shaped and 7.31% were of alveolar type. Papillary type pituitary adenomas registered the highest intensity of expression for protein S100 in tumor cells (fig. 4a). With the exception of papillary type adenomas, in the majority of pituitary adenoma cases the intensity of the reaction was low and moderate, in comparison with the normal tissue (fig. 4b).

The acidophilic cells were present in percentage of 34.2% of cases. Pituitary adenomas with basophilic cells represented percentage of 26.8% of positive cases for protein S100, and, in 39% of cases the chromofobe component was present forming pure chromofobe pituitary adenomas or mixed chromofobe-acidophilic/basophilic pituitary adenomas.

Regarding the particularities of the immunohistochemical expression of protein S100, we observed variabilities in the presence, intensity and distribution of this marker in relation to the hormone profile. Growth hormone secreting pituitary adenomas proved to be extremely hetherogenous in what is considered the expression of protein S100 in tumor cells. The cases ranged from the absence of its expression in tumor cells (stellate follicular cells being positive, fig. 5 a) to a low expression, strictly located in the cytoplasm (fig. 5 b), an intense expression, in the entire tumor area, nuclear and cytoplasmic (c) or just cytoplasmic (d).

In case of pituitary adenomas with chromofobe cells, the expression of protein S100 was low in the cytoplasm of the chromofobe cells, being mostly nuclear restricted (fig. 6).

Tumor cells were negative for protein S100. On the other

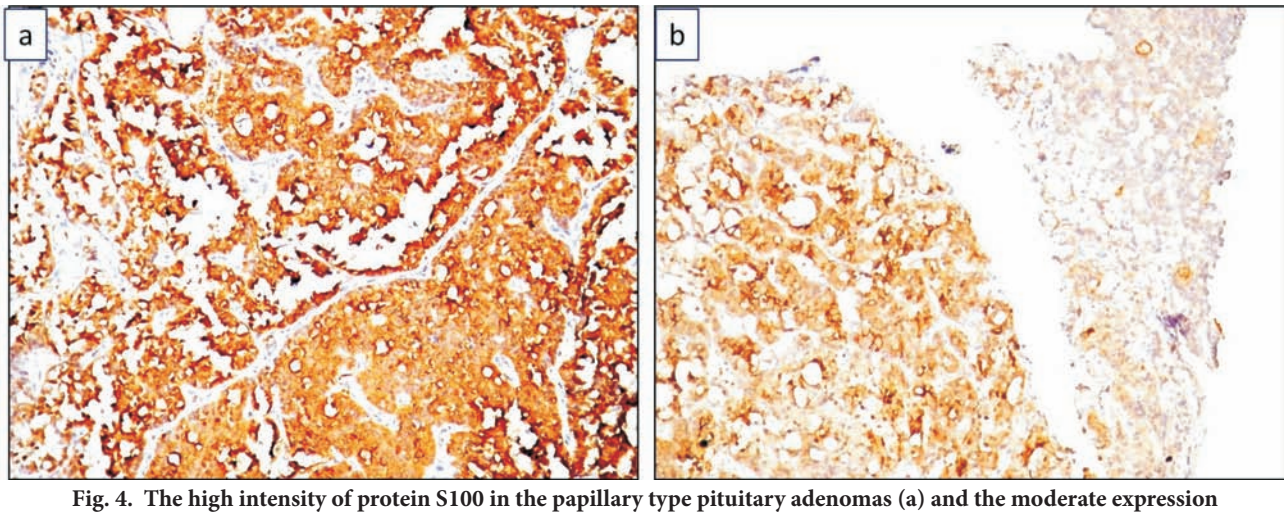

Fig. 4. The high intensity of protein S100 in the papillary type pituitary adenomas (a) and the moderate expression in the adenomatos tissue (b, right section) compared to the normal pituitary gland (b, left section).

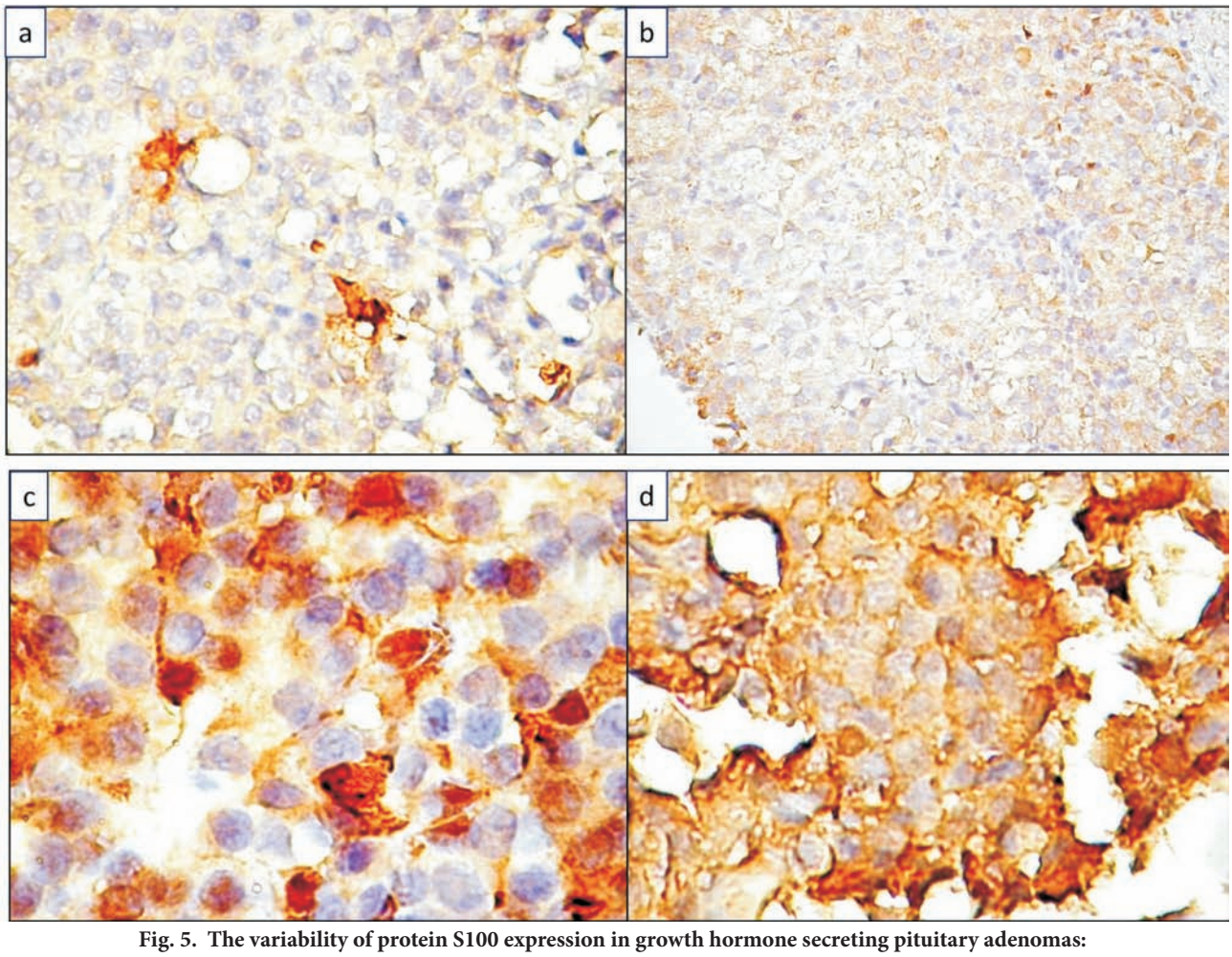

Fig. 5. The variability of protein S100 expression in growth hormone secreting pituitary adenomas: absent (a), low (b), intense, nuclear and cytoplasmic (c), intense, cytoplasmic (d).

hand, around the vascular spaces, S100 positive cells were distributed, having a nuclear and cytoplasmic expression, and morphology similar to that of stellate follicular cells. The expansions of the stellate follicular cells were strongly attached to the wall of the blood vessel, being also interconnected with one another (fig. 7a). These interconnections created a network of S100 positive expansions amongst which tumor cells were distributed. Focally, in the immediate vicinity of the S100 positive cells, tumor cells presented a low and inconstant reaction with a strict cytoplasmic distribution (fig. 7b).

As in the case of growth hormone secreting pituitary adenomas, the endothelial cells of the vessels located in the vicinity of the S100 positive cells had a positive reaction for protein S-100 with a nuclear distribution (fig. 7c). In the rest of

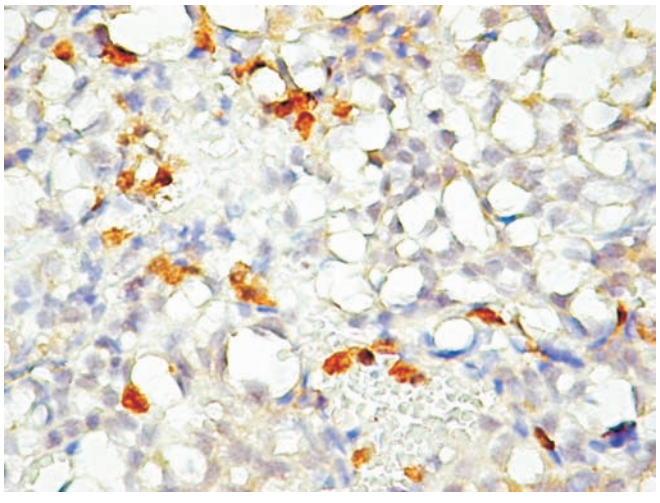

Fig. 6. Chromofobe type pituitary adenoma with chromofobe cells negative for protein S100 in their cytoplasm, with a focal and hetherogenous expression in the nuclei of the chromofobe cells and in the cytoplasm and the nucleus of stellate follicular cells inserted amongst chromofobe cells. In this case also, we may note the presence of endothelial cells that have a nuclear positive reaction for protein S100.

the tumor mass the S100 positive cells with a stellate follicular morphology were rarely encountered in adrenocorticotropic hormone (ACTH) secreting pituitary adenomas.

In relation to the hormone profile, none of the six markers used for immunohistochemical profiling significantly correlated from a statistical point of view with the expression of protein S100.

Discussion

Protein S100 expression was studied in pituitary adenomas, being frequently associated with stellate follicular cells [1, 2]. Their expression in tumor cells, separately quantified in various types of pituitary adenomas represents a sporadic subject in literature and, due to this reason; the correlations with the prognosis, long term survival, recurrences and therapeutic implications are extremely unconvincing at the time being. Increasing data suggest the capacity of tumor endocrine and non-endocrine cells to transdifferentiate themselves in stellate follicular cells where the two markers overlap in what is considered their immunohistochemical expression, these

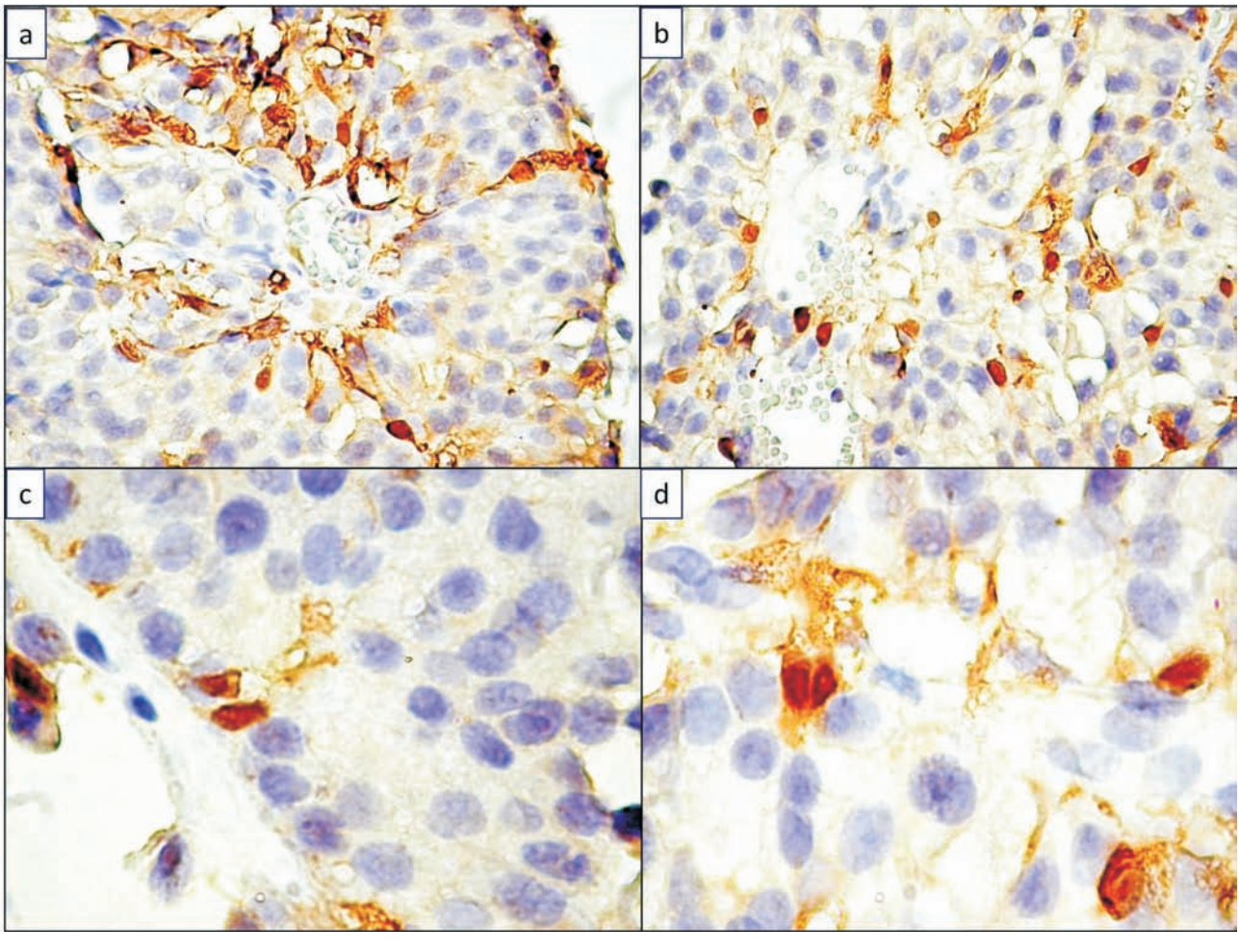

Fig. 7. Protein S100 is expressed in ACTH secreting pituitary adenomas. S100 positive cells, at a nuclear and cytoplasmic level, distributed in palisade around the blood vessels (a). These cells present numerous branched expansions, inserted amongst tumor cells, on the one hand and interconnected with one another forming networks (b, c). On occasion, S100 positive cells were observed as groups located amongst tumor cells that presented a low focal reaction for protein S100 (b, d).

FS cells actually being considered as pluripotent stem cells [3, 4]. The "retrodifferentiation" phenomenon was observed and described especially in case of ACTH secreting pituitary adenomas [4]. In our study we also observed a particular aspect of distribution, localization and S100 expression in case of ACTH secreting pituitary adenomas.

As a paradox, the description of protein S100 expression in the normal pituitary gland is restricted only at the evaluation of foliculostimulating hormone cells. We have not found any data in the literature regarding protein S100 expression, differentiated in the endocrine cells of the normal human pituitary gland. We observed a moderate immunohistochemical expression in the acidophilic, growth hormone secreting cells, and low in the chromofobe cells. The expression pattern also remained in case of growth hormone secreting pituitary adenomas where we noticed a variability of S100 expressions. This aspect was visible especially in case of mixed pituitary adenomas where the growth hormone secreting component was intensely positive for protein S100 while the basophilic component was negative or the chromofobe one was low positive for protein S100.

Conclusions

1. Protein S100 expressions in tumor cells is implicated in the pathogenesis of the growth hormone and prolactin secreting pituitary adenomas, the mechanisms of activation being nowadays incompletely studied. This aspect seems to represent an unfavorable prognostic factor that governs the retrodifferentiation phenomenon and supports the presence of pluripotent stem cells.
2. Through analogy with the observations obtained in other tumor types, it is possible that protein S100 pituitary adenomas to represent a group of pituitary adenomas with an aggressive behavior and a high capacity of invasion and recurrence, aspects that represent an unfavorable prognostic factor.

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Structural peculiarities of the maxilla and its surfaces in the perinatal period of ontogenesis

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Abstract

Background: Congenital clefts of the upper lip and palate are not more often as a part of this or that syndrome, but as an independent congenital disease in the form of an isolated developmental defect of separate organs. Therefore, it is necessary to detect and systematize peculiarities of the development and structure of the maxilla and its body in the perinatal period of ontogenesis.
Material and methods: The study was conducted on 53 dead 4-10-month fetuses and 11 newborns (5 isolated organ complexes in particular) of both sexes without external signs of anatomical defects or abnormalities and without vivid macroscopic deviations from the normal structure of the skull. Before the beginning of the craniometric examination every specimen was fixed in craniostat in the horizontal auricular-ocular plane, in so-called "Frankfurt horizontal line". All the measurements on the skulls were made by means of a tape measure, caliper, slide compasses and dial calipers.
Results: With the age of fetuses a short and wide shape of the upper jaw changes into a high and narrow one. The absence of the zygomatic-cellular crest is a characteristic sign of the fetuses of all the age groups and newborns. During perinatal period of ontogenesis infraorbital opening is usually projected in the point of crossing of the line connecting a lateral angle of the eye with the wing of the nose and the line passing from the medium angle of the eye to the angle of the mouth.
Conclusions: A typical shape of the maxilla during the perinatal period is short and wide found in early fetuses (4-5 month) – in 94% of cases, in fetuses of 6-7 months of age – in 82% and in fetuses of 8-10 months of age (late fetuses) – in 68% and newborns. A typical shape of the anterior surface of the maxilla for early fetuses is irregular trapeziform, and for 6-7-month fetuses, late fetuses and newborns – an elongated triangle shape.
Key words: fetus, maxilla, infraorbital opening, ontogenesis.

Introduction

According to statistical data of the Ministry of Public Health (MPH) of Ukraine occurrence of congenital defects among children at the age under 17 in Ukraine for the recent decade has a convincing tendency to increase from 19.49% to 26.7% [5].

Congenital defects of the muscular-skeletal system occupy the second place among all the congenital developmental defects [6]. Every year in Ukraine over 600 children are born with congenital developmental defects of the maxillary-facial area including 400-450 cases with congenital cleft of the upper lip and palate [3, 5]. Congenital clefts of the upper lip and palate are not most often a part of this or that syndrome, but as an independent congenital disease in the form of an isolated developmental defect of separate organs [7, 8]. Availability of comorbid dental pathology requires mutual efforts and cooperation of both theoretical and practical medical men of various specialties to introduce a complex of measures directed to the prevention and timely diagnostics of occurrence of dental-maxillary defects as well as improvement of dental health of children [1, 2, 4].

Therefore, insufficient and fragmentary study of the evidence of structural peculiarities of the maxilla and its body, peculiarities of the formation of the shape and topographic-anatomical interrelations of the upper jaw in the perinatal period of ontogenesis require a careful investigation.

Material and methods

The study was conducted on 53 dead 4-10-month fetuses and 11 newborns (5 isolated organ complexes in particular) of both sexes without external signs of anatomical defects or abnormalities and without vivid macroscopic deviations from the normal structure of the skull. The study was conducted according to the methodical recommendations "Keeping to ethical and legal standards and requirements in making scientific morphological studies" and followed the main directions of Helsinki declaration of the World Medical Association concerning ethical principles to conduct scientific-medical investigations with human participation (1964-2000) and the Order of the MPH of Ukraine dated 23.09.2009 № 690.

The organ and cranial metric results were analyzed on common craniometric points, distances between them, and along the main special planes and lines according to the Recommendations on Anthropologic and Medical Craniology (V. P. Vorobyov, 1932; V. N. Shevkunenko, 1947; V. V. Bunak, 1941, 1953; Y. Y. Roginskyi, M. G. Levyn, 1955; V. P. Alekseyev, G. F. Debets, 1964; V. G. Koveshnikov, 1965; V. S. Speranskiy, 1991). Before the beginning of the craniometric examination every specimen was fixed in craniostat in the horizontal auricular-ocular plane, in so-called "Frankfurt horizontal line". All the measurements on the skulls were made by means of a tape measure, caliper, slide compasses and dial calipers.

Results and discussion

On the basis of the analyzed results of our own study we can state that in early fetuses (4-5 month) – in 94% of cases, in fetuses of 6-7 months of age – in 82% and in fetuses of 8-10 months of age (late fetuses) – in 68% and newborns a typical shape of the maxilla is short and wide. A short and wide shape of the maxilla changes into a high and narrow one with age. In 4-5-month fetuses a high and narrow shape of the maxilla is 6%, in 6-7-month fetuses – 18% and in 32% of late fetuses (8-10 month) and newborns (fig.1).

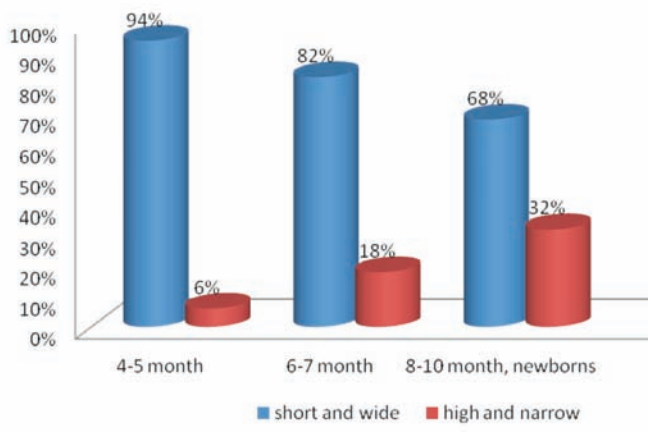

Fig. 1. Variants of the maxillary shape.

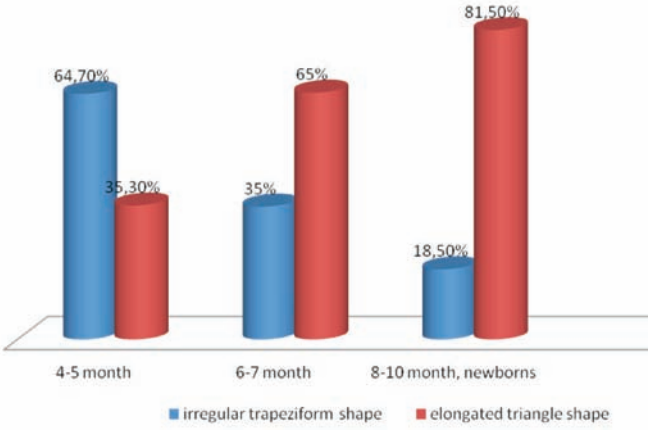

Fig. 2. Structural variants of the anterior surface of the maxilla.

A typical shape of the anterior surface of the maxilla for early fetuses (4-5 month) is irregular trapeziform in 64.7%, and for 6-7-month fetuses – 35%, late fetuses (8-10 month) and newborns – in 18.5%. In 35.3% of 4-5-month fetuses, in 65% of 6-7-month fetuses and in 81.5% of 8-10-month fetuses and newborns the anterior surface of the maxilla becomes of an elongated triangle shape.

It is indicative of the fact that a typical shape of the anterior surface of the maxilla for early fetuses is irregular trapeziform, and for 6-7 month, late fetuses and newborns – an elongated triangle shape (fig.2). In the perinatal period of ontogenesis all the objects of the study had a rough surface of the maxilla formed of a spongy substance.

With the age of fetuses the relief of the anterior surface of the maxilla changes. Thus, a flat anterior surface of the maxilla is found in 4-month fetuses, it changes into a little concave one in the area of the infraorbital opening in 5-month fetuses. In 6-7-month fetuses the surface is more concaved passing from the base of the frontal process to the infraorbital opening. In 8-10-month fetuses and newborns a deep concavity is found near the cellular process from the nasal incisures to infraorbital opening. In the perinatal period of ontogenesis the height of the anterior surface increases by 2,3 times, and the length – by 2,1 times as much. The height and length of

the anterior surface of the maxilla increase most intensively in 8-10-month fetuses and newborns, and the slowest – in 5-month fetuses of the intrauterine development.

The absence of the zygomatic-cellular crest is a characteristic feature of fetuses in all the age groups and newborns. Therefore, the anterior surface of the maxilla without clear borders passes into the infratemporal one. The border between these two surfaces is a projection extension of the base of the zygomatic process to the cellular process. In all the fetuses and newborns infratemporal surface is prominent, rough, and directed from the anterior surface dorsally and parallel to the median plane and upwards. Externally and upwards it is covered by the zygomatic bone and zygomatic process of the maxilla. In the perinatal period of ontogenesis the height of the infratemporal surface becomes by 2,3 times as much, and its length – by 1,7 times as much. A considerable increase of the height and length of the infratemporal surface of the maxillary body occurs in 8-10-month fetuses and newborns, and it is the slowest in 5-month fetuses. The ratio of the height of the anterior surface to the height of the infratemporal surface in the perinatal period is in an average 1:1 (1:1,03 – in 5-month fetuses and 1:1,25 – in 6-month fetuses), which is indicative of the similarity of the height sizes of these surfaces. The ratio of the length of the anterior surface of the maxillary body and the length of the infratemporal surface in the perinatal period ranges between 3,1:1 (in 4-month fetuses) and 4,2:1 (in 8-10-month fetuses), which is indicative of a considerable development of the anterior surface in its length which is connected with the development of the cellular process.

During the study the infraorbital opening was found in all the objects. In all 4-5-month fetuses the infraorbital opening is oval in its shape and is located in the center of the anterior surface of the maxillary body. In 15% of 6-7-month fetuses this opening becomes round, and in other 85% – it is oval. During this period the infraorbital opening is shifted upwards and located in the center in the upper third of the anterior surface. Beginning with the 8th month of the intrauterine development the infraorbital opening moves laterally and is located in the center between the median and distal third of the anterior surface of the maxillary body and preserves its oval shape. An oval shape of the infraorbital opening should be considered a typical one, and a round one – as a variant of it.

During perinatal period the sizes of the infraorbital opening range from 0,7×0,6 mm (in 4-month) to 2,0×1,2 mm (in newborns). The distance from the center of the infraorbital opening to the orbit border of the same name is on average 1,5-1,7 mm in 4-month fetuses; 1,8-2,0 mm in 5-month fetuses; 1,8-2,2 mm in 6-month fetuses; 2,1-2,4 mm in 7-month fetuses; 1,8-3,0 mm in 8-10-month fetuses and 2,5-4,0 mm in newborns. The direction of the infraorbital opening passes upwards and from the periphery to the median plane.

During the perinatal period of ontogenesis the infraorbital opening is usually projected in the point of crossing of the line connecting the lateral angle of the eye with the nasal wing and the line passing from the median angle of the eye to the angle of the mouth. In early (4-5-month) fetuses this projection of the infraorbital opening is found in 70.6% – in the right and 64.7% – in the left, in 6-7-month fetuses in the right – in 75% and in the left – 80%, and in late fetuses (8-10-month) and newborns – in 74% and 77.7% respectively.

At the same time, during the study different variants of location of the infraorbital opening were found. This opening was located higher or lower of the typical projection 0,3-0,5 mm to the right, and 0,4-0,8 mm to the left. In 4-5-month fetuses a variant projection was found in the right in 29.4% (23.52% higher and 5.88% lower) and in the left – in 35.3% (23.52% higher and 22.76% lower), in 6-7-month fetuses: in the right – in 25% (15% higher and 10% lower) and in the left – in 20% (15% higher and 5% lower), and in 8-10-month fetuses and newborns in 26% (18.5% higher and 7,4% lower) and 22.3% (14.9% higher and 7,4 lower) respectively. In the variant structure the location of the infraorbital opening higher of the typical projection is found more frequently (fig. 3 and fig.4).

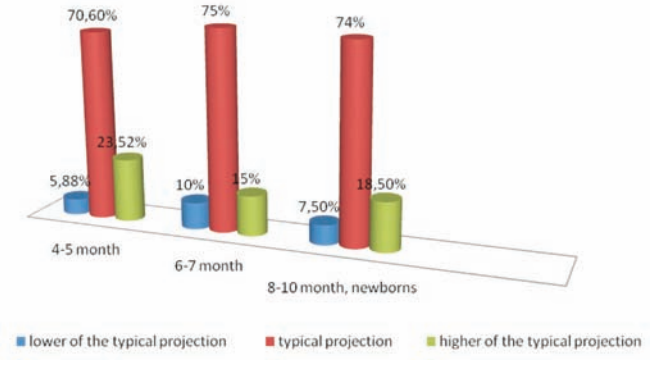

Fig. 3. Projection of the infraorbital opening on the right upper jaw.

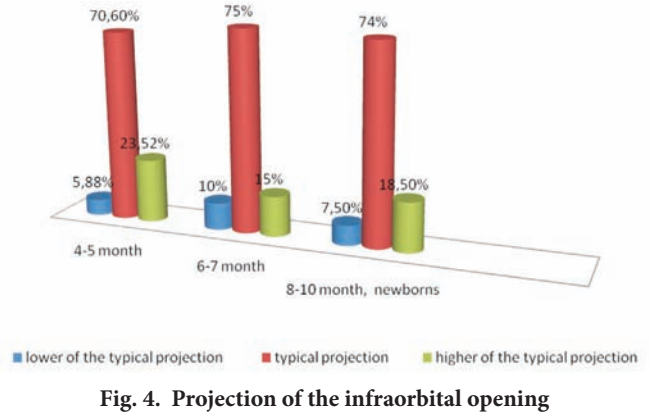

Fig. 4. Projection of the infraorbital opening on the left upper jaw.

Conclusions

1. A typical shape of the maxilla during the perinatal period is short and wide and is found in early fetuses (4-5 month) – in 94% of cases, in fetuses of 6-7 months of age – in 82% and in fetuses of 8-10 months of age (late fetuses) – in 68% and newborns. A short and wide shape of the maxilla changes into a high and narrow one with age.

2. A typical shape of the infraorbital opening is oval, and round is considered to be as a variant of it. During the peri-

natal period of ontogenesis the infraorbital opening is usually projected in the point of crossing of the line connecting the lateral angle of the eye with the nasal wing and the line passing from the median angle of the eye to the angle of the mouth. In early (4-5-month) fetuses this projection of the infraorbital opening is found in 70.6% – in the right and 64.7% – in the left, in 6-7-month fetuses in the right – in 75% and in the left – 80%, and in late fetuses (8-10-month) and newborns – in 74% and 77.7% respectively.

3. A typical shape of the anterior surface of the maxilla for early fetuses is irregular trapeziform, and for 6-7 month, late fetuses and newborns - an elongated triangle shape. The ratio of the height of the anterior surface to the height of the infratemporal surface in the perinatal period is on average 1:1 (1:1,03 – in 5-month fetuses and 1:1,25 – in 6-month fetuses), which is indicative of the similarity of the height sizes of these surfaces. The ratio of the length of the anterior surface of the maxillary body and the length of the infratemporal surface in the perinatal period ranges between 3,1:1 (in 4-month fetuses) and 4,2:1 (in 8-10-month fetuses), which is indicative of a considerable development of the anterior surface in its length which is connected with the development of the cellular process.

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REVIEW ARTICLES

Echinacea compositum in the treatment of respiratory diseases

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Received March 14, 2016; accepted April 05, 2016

Abstract

Background: Clinical protocols and standards of treatment of respiratory tract diseases include mainly antibacterial, mucolytic and anti-inflammatory therapy. This approach is effective at relieving acute symptoms, but there is a "flip side of the coin" – presence of side effects. So, antibiotics cause immunosuppression and dysbiosis, nephrotoxicity and hepatotoxicity. Nonsteroidal anti-inflammatory medicines have a negative effect on mucous membrane of stomach and kidneys. Mucolytics, beta-2-agonists increase blood pressure, cause heart rhythm disturbances. In case of a more long-term use of these medicines these problems become more relevant. Thus, there is a shortage of pathogenic anti-inflammatory therapy aimed at optimization of inflammation. In standards are absent also effective drainage and detoxification medicines and impossibility of their use in comorbidity, age-related limitations also complicates the treatment.

Conclusions: Echinacea compositum has a complex effect: detoxification, immunomodulatory, anti-inflammatory, etc. Medication is used in treatment of inflammatory and purulent processes of soft tissues and mucous membranes; its prescription does not require prior immunological examination. It has a favorable safety profile and is recommended for children from 1 year old, is well combined with any medicine, increases nonspecific protection, effectiveness of antibiotic therapy and course of treatment, reduction of its duration; enhances antifungal therapy and is used in the schemes of influenza prevention in children, the elderly, patients with tendency to allergic reactions.

Key words: Echinacea compositum, bioregulation, respiratory tract.

Introduction

Proportion of acute and chronic diseases of the upper and lower respiratory tract infections among all newly diagnosed diseases is 26% [2]. They significantly reduce quality of life of patients, are dangerous with high risk of chronicity and development of complications, especially metagrippal [1]. Here is presented the review of long-term foreign clinical experience of the use of complex bioregulation medication Echinacea compositum of German company "Biologishe Haylmittel Heel" for diseases of respiratory tract [4, 5, 6].

Clinical protocols and standards of treatment of respiratory tract diseases include mainly antibacterial, mucolytic and anti-inflammatory therapy. This approach is effective at relieving acute symptoms, but there is a "flip side of the coin" - presence of side effects. So, antibiotics cause immunosuppression and dysbiosis, nephrotoxicity and hepatotoxicity. Nonsteroidal anti-inflammatory medicines have a negative effect on mucous membrane of stomach and kidneys. Mucolytics, beta-2-agonists increase blood pressure, cause heart rhythm disturbances. In case of a more long-term use of these medicines these problems become more relevant. [3]

More and more often appears resistance of microorganisms to antimicrobial agents, and the growth of population sensitization limits possibilities to prescribe standard medicines [3]. Nonsteroidal anti-inflammatory medicines have mainly symptomatic effects on inflammatory process by blocking and suppressing it. Thus, there is a shortage of pathogenic anti-inflammatory therapy aimed at optimization of inflammation. In standards are absent also effective drainage and detoxification medicines and impossibility of their use in comorbidity, age-related limitations also complicates the treatment.

Discussion

In connection with it expansion of the use of pathogenetic bioregulation approaches and medications is important as it can improve both efficiency and profile of therapy safety. One of such approaches is bioregulation. It is carried out through the use of integrated bioregulation medicine. Earlier in the literature was used the term "antihomotoxic medications". Complex bioregulation medications contain ultralow doses of active substances, which contribute to the activation of drainage and detoxification processes, recovery of self-control processes in the body (acute inflammation and others.). Ultralow doses of complex bioregulation medication are not metabolized in the body and do not have pharmacokinetics. They do not require additional energy and do not have pharmacological load on the body [4, 5, 6].

Complex bioregulation medicine has a pronounced detoxification, immune-modulating and anti-inflammatory effect – Echinacea compositum (injectable solution). It is used in complex treatment of inflammatory and purulent processes of soft tissues and mucous membranes, and particularly in cases with severe intoxication and frequent recurrences [1, 2, 3, 9, 12, 14].

In the National Medical University (Kiev, Ukraine) S. A. Kramarev, L. A. Palatnaya, B. K. Shamugia [7] developed methodical recommendations "Alternative methods of treatment and prevention of influenza and acute viral infections in children" where Echinacea compositum is regarded as a universal immunomodulator, which can be administered without prior immunological examination that essentially simplifies the work of family doctor.

In the Institute of Tuberculosis and Pulmonology of the National Academy of Medical Sciences of Ukraine V. P. Kostromina, L. B. Yaroshchuk [9] conducted an open, randomized research "Efficacy of antihomotoxic medications in the treatment of recurrent bronchitis in children infected with mycobacterium of tuberculosis". The efficiency of CBM Echinacea compositum, Limfomiozot (drainage medication, which improves lymph drainage) and 2 others were studied. The control group received standard therapy. The second, in addition Limfomiozot and Mucosa compositum. The third, only complex bioregulation medications: Limfomiozot, Echinacea compositum, Mucosa compositum and Traumeel.

Made conclusions: monotherapy with complex bioregulation medications / antihomotoxic medications in their effectiveness is not inferior to the standard scheme of therapy of recurrent bronchitis; complex bioregulation medications (antihomotoxic medications significantly reduce the level of endotoxemia) have a significant normalizing effect on the structure and function of mucous membranes of the respiratory and digestive tracts and state of microflora [9].

In the research of L. M. Senyuta, G. I. Mazuryak, A. L. Tsimbalist, S. S. Moldaver [10] "Therapeutic efficacy and tolerability of Echinacea compositum preparation, at various stages of treatment of severe pneumonia in infants", conducted on the basis of Regional Children Clinical Hospital in Ivano-Frankivsk city (Ukraine) were involved 46 children between the ages of 1 month to 1 year.

To the first group on the background of basic traditional therapy was prescribed CBP Echinacea compositum. Compared with control group (only basic therapy) the first group manifested a significant improvement of general state, reduction of the duration of intoxication, obstruction, fever, respiratory distress, more rapid normalization of blood formula and reduction of the duration of course of antibiotic therapy. Finally, have been made conclusions that parenteral use of Echinacea compositum in the treatment of acute viral and bacterial pneumonia enhances the effectiveness of antibiotic therapy and normalization of humoral response; it can be recommended for infants for pneumonia therapy, for prevention – to children from group at high risk of pneumonia development [10]. As a result, by the order No. 18 of January 13th, 2005 of the Ministry of Health of Ukraine, complex bioregulation medication Echinacea compositum is included in clinical protocol of the treatment of staphylococcal pneumonia in children [11].

In methodological recommendations "Principles of etiopathogenic therapy of acute pharyngitis", S. V. Ryazantsev and V. I. Kocherovets [12] characterized Echinacea compositum as "biological antibiotic" and noted its high efficacy against infectious processes of staphylococcal and streptococcal etiology.

Echinacea compositum greatly enhances antifungal therapy in composition of complex treatment of mycoses – methodological recommendations "Clinics and treatment of mycotic lesions of upper respiratory tract and ear". At uncomplicated course of mycoses (invasive forms) complex bioregulation medications may be administered as monotherapy, and at presence of comorbidity - simultaneously with standard treatment of mycoses of upper respiratory tract and ear [13].

In the National Medical University (Kiev, Ukraine) P. F. Dudka, I. I. Saharchuk, R. I. Ilnitsky [14] developed methodological recommendations "Antihomotoxic medications in the treatment of chronic obstructive pulmonary disease". In this case Echinacea compositum is recommended as a basic complex bioregulation medication in the scheme of chronic obstructive pulmonary disease treatment. Besides immunomodulatory action focus is made on its strong anti-inflammatory, indirect antimicrobial, detoxification and drainage action.

Conclusions

The use of complex bioregulation medications is pathogenetically substantiated method of chronic obstructive pulmonary disease treatment. Both in exacerbation and steady state of patient, they enhance clinical efficacy of chronic obstructive pulmonary disease treatment, reduce the dosage of antibiotics, bronchodilators, inhaled corticosteroids and lower incidence of their side effects; in case of non-severe exacerbation of chronic obstructive pulmonary disease, caused by respiratory viruses, it is possible only the use of complex bioregulation medications/ antihomotoxic medications.

Echinacea compositum has a complex effect: detoxification, immunomodulatory, anti-inflammatory, etc. Medication is used in treatment of inflammatory and purulent processes of soft tissues and mucous membranes; its prescription does not require prior immunological examination. It has a favorable safety profile and is recommended for children from 1 year old, is well combined with any medicine, increases nonspecific protection, effectiveness of antibiotic therapy and course of treatment, reduction of its duration; enhances antifungal therapy and is used in the schemes of influenza prevention in children, the elderly, patients with tendency to allergic reactions.

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IN MEMORIAM

Vasilii Chekurin – 125 years since the date of the birth

The XXth century was so dynamic that it could not but influence the biographies of those who lived and worked during those changeable times. 72 years of Professor Chekurin were full of intensity and anxiety: he was born during the rule of Tsar Alexander the IIIrd, experienced revolutions and wars, famine, technological base change and development and left this world in the era of space flights.

Vasilii Chekurin was born on April, 20th 1891 in Petrovsk city, Saratov province in a family of peasants. He was the first in his family who graduated from a University, received Diplomas of doctor of medical sciences and professor.

The future professor studied medicine in Universities of Saratov and Permi. He received his diploma of a doctor in 1923. His practical and scientific experience is valuable and useful until our days.

When a student he was interested in scientific works of Professor Leonid Komendantov, he worked in Otorhinolaryngology (ORL) Department as a clinical intern and assistant. After graduation in 1923 he was a scientific researcher of ORL Department, under the supervision of Professor Komendantov. He continued research in the University of Rostov-on-Don, organized and headed the ORL office and otorhinolaryngology department at the Petrovsk county hospital and the ORL department in Novocherkassk city.

Practical and administrative work Professor Chekurin combined with outstanding scientific activity. In 1926 there was published his first scientific article: "About nasal septum tuberculosis", in the journal Messenger of Otorhinolaryngology, No 6.

A new stage of his scientific activity was dedicated to theoretical basic elements and experimental work regarding the rehabilitation of balance disturbances. He succeeded in elaborating a serious experimental work in a short period.

In 1931, V. Chekurin becomes assistant in Leningrad Institute of Children and Teenagers' Health Protection and assistant of ORL department of the first Medical Institute of Leningrad. Due to the great number of scientific works, Doctor Vasillii Chekurin was awarded PhD degree in medical sciences (candidate of medical sciences) in 1938.

One year later, in 1939, the original experimental research of Dr. Chekurin resulted in his second PhD degree in medical sciences thesis (doctor of medical sciences) for his scientific thesis "Compensation function of statokinetic receptors at the disturbance of vestibular apparatus".

During his activity in Leningrad Medical Institute he was concerned about ear physiology and pathology problems, as well as rehabilitation of hearing and speech in deaf–mute schools. Dr. Chekurin was the first who organized special classes for children with hearing problems, where didactical teaching was carried out parallel with hearing rehabilitation. In Leningrad, Dr. Chekurin published 14 scientific articles and booklets about treatment of deaf children.

Doctor's work and further activities of Professor Chekurin proved his organizational capacities. Professor Chekurin participated in founding of ORL clinics in Rostov-on-Don and Leningrad. He led the ORL department in Tashkent Institute of Medicine, at the same time he was the deputy commissioner on health problems in the Soviet Socialist Republic of Uzbekistan, deputy head of education department of the Central Committee of the Communist Party of Uzbekistan. Professor Chekurin was engaged in curative, teaching and administrative activities.

During his activity at the ORL department of the Institute of Medicine in Tashkent, Professor Vasilii Chekurin paid a special attention to the problem of nervous supply disorders in ORL diseases and developed nontraumatic methods and conservative techniques in treatment of patients with severe ORL pathologies. For example, he elaborated and introduced the techniques so as to remove foreign bodies from children's respiratory organs, using exclusively the functional endoscopic approach, thus excluding the need for traumatic tracheotomy.

In 1945 he was appointed the head of ORL department of Leningrad Medical Institute that was at that time evacuated to Kislovodsk and later transferred to Chisinau. Thus, in 1945 Professor Chekurin founded the Department of Otorhinolaryngology in the Institute of Medicine of Chisinau and laid the basis of scientific researches of ORL related issues in Moldova.

In Chisinau Institute of Medicine Professor Chekurin succeeded in accomplishing a great part of his ideas. He published a monograph and organized the work for preven-

ting ORL pathologies in vulnerable regions of the Republic of Moldova. Professor Chekurin founded the Association of Otorhinolaryngologists in Moldova. Practitioners were meeting at seminars organized by Professor Chekurin, where the most interesting clinical cases were presented and analysed.

In 1947 the Moldovan State Publishing House issued the book of Professor Chekurin "Balance restoration after vestibular apparatus disturbance". This was one of the first books published by the order of Chisinau Medical Institute, having 10 printed sheets and a circulation of 2 thousand copies. After half a year, the book was submitted to the Stalin Prize, the highest distinction at that time.

In 1951, Professor Vasilii Chekurin was transferred to the Institute of Medicine in Ryazani, Russia, but he contributed a lot for his students of Chisinau Institute of Medicine M. Zagarskih and M. Sandul to receive their PhD degrees in medical sciences. The doctors of the first two graduations of Chisinau Medical Institute continued the work of Professor Chekurin in medical systems of both Moldova and other countries.

In the assembly hall of Scientific Council of Nicolae Testemitsanu State University of Medicine and Pharmacy is hanging up to now the portrait of Professor Vasilii Chekurin, who jointly with other scientists and doctors have created under difficult conditions the Medical Institute in Chisinau.

Professor Chekurin's spectrum of scientific research was very varied and vast. His research themes, which were elucidated in leading scientific journals and presented as reports in frame of various congresses and conferences contained the most current issues in otorhinolaryngology field.

Professor Vasilii Chekurin studied deeply and consistently the otology and audiology problems, namely, deafness and hearing loss, the impact of industrial noise on auditory organs of working teenagers, etc.

Children's ORL pathology of organs and especially of those with impaired hearing occupied a special place among the works of Professor Chekurin. Scientific researches served as basis for publications, reports, papers, both for organizational work with children with hearing problems and scientific investigations of the causes of hearing loss and methods of rehabilitation of the little patients.

Vasilii Chekurin was awarded numerous distinctions including for founding the State Medical Institute in Chisinau, for outstanding achievements in scientific and teaching process organization and active participation in the scientific work.

In 1949, by the decision of the Presidium of the Supreme Council of the USSR, Vasilii Chekurin was awarded the Order "Insignia of Honour" for longevity in activity and irreproachable work within higher educational institutions.

Ababii Ion, MD, PhD1, PhD2, Professor, Academician
Chairman of the Department of Otorhinolaryngology
Rector of Nicolae Testemitsanu State University
of Medicine and Pharmacy.

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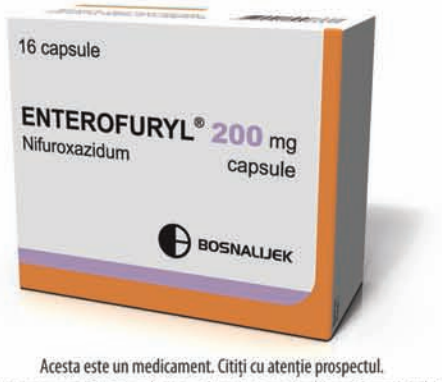

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3. The abstract should be written on the title page in English and be limited trom 220 to 240 words. The abstract should end with 3 to 6 key words.

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 no more than 40 References. The **review articles** must not exceed 25 pages and contain no 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 colour. If coloured 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. The references in the Cyrillic script should be transliterated into Latin script as follows: А–A, Б–B, В–V, Г–G, Д–D, Е–E, Ё–E, Ж–ZH, З–Z, И–I, Й–Y, 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–SCH, Ы–Y, Э–E, Ю–YU, Я–YA, Ь and Ъ are omitted. Immediately after the transliteration the translation of the title in English [in the square brackets] should follow. For example: Ivanov IV, Sidorov VM, Kozlov NF. Transplantatsiya organov i tkaney [Transplantation of organs and tissues]. Vestnik Khirurgii [Messenger of Surgery]. 2010; 26(6):45-49.

GHID PENTRU AUTORI

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3. Rezumatul în limba engleză (220-240 cuvinte) se expune consecutiv pe foaia de titlu, inclusiv cuvintecheie, de la 3 până la 6. În rezumat este obligat să fie expus scopul cercetării (dacă nu este clar din titlu), metodologia studiului, rezultatele obţinute şi concluziile.

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ГИД ДЛЯ АВТОРОВ

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