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RESEARCH STUDIES

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Anti-inflammatory activity of Adenoprosin in nonbacterial prostatitis

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Abstract

Background: The treatment of chronic nonbacterial prostatitis remains an unexplained urology problem. Adenoprosin is a new entomological product containing lipoprotein extract of Lepidopteran insect species. Laboratory studies of the product have shown that it possesses antioxidant, antiproliferative and anti-inflammatory properties.

Material and methods: Nonbacterial prostatitis was experimentally modeled on 100 white Wistar rats. Adenoprosin, 150 mg rectal suppositories, was tested against the reference product Vitaprost, 50 mg rectal suppositories and placebo on both the aseptic acute and chronic non-bacterial prostatitis models. In the acute prostatitis model, treatment lasted 7 days and in chronic prostatitis 15 days. The treatment efficacy criteria consisted of assessment of the general condition and histological results.

Results: In rats receiving Adenoprosin, the microscopic image of the prostate showed a decrease in the severity of the inflammatory process, in both acute aseptic and chronic nonbacterial prostatitis, manifested by the recovery on the surface of the epithelial cells in the stromal area of the prostate, and decrease in vascular congestion and number of acini with desquamated epithelium.

Conclusions: The product of entomological origin Adenoprosin, showed an obvious anti-inflammatory effect in the experimental model of aseptic acute or chronic non-bacterial prostatitis induced on Wistar white rats, similar to the Vitaprost reference product, and significant compared to placebo ($p<0.05$).

Key words: Adenoprosin, prostatitis model, non-bacterial prostatitis treatment.

Introduction

Prostate diseases have been and continue to remain a major and controversial issue in urology. Prostatitis is the acute or chronic inflammation of the prostate gland and is characterized by the presence of pain in the groin, pelvic area or genitals, frequent urination, dysuria (difficulty urinating or burning sensation when urinating), and in acute inflammation flu-like symptoms. The persistence of prostate symptomatology has a negative impact on patient's sexual life and quality of life [1, 2, 3, 4].

The controversy begins with prevalence studies and ends with treatment methods. The prevalence of chronic prostatitis in the general population fluctuates from 10 to 38%, and that assessed by using validated NIH-CPSI questionnaires proposed by USA National Institute of Public Health, from 10 to 14% [5, 6, 7].

Though thousands of articles have been published, some clinicians tend to underestimate the illness and its consequences, and others to overdiagnose, especially attributing sexual complaints. Other controversies also arise in terminology; the term prostatitis involves the presence of an inflammatory disease, whereas bacterial infection itself is present in prostatitis patients in only 10% of cases. Approximately 40-60% of patients will have leukocytes and no bacteria, and the remaining 30-40% will have chronic aseptic prostatitis, prostatodynia, or chronic painful pelvic syndrome.

Without a clear classification of chronic prostatitis, treatment methods remain controversial and often uncertain. The role of antibacterial therapy is well defined for acute or chronic bacterial prostatitis, and uncertain for aseptic prostatitis and chronic pelvic pain syndrome [8, 9, 10].

For a deeper study of the causes of nonbacterial prostatitis, and their testing various treatment methods have been proposed among them several experimental models of prostatitis, that mimic human chronic prostatitis phenotype. The closest model is the one induced on white rats. Aseptic prostatitis can be induced by several methods, such as direct injection into the rat prostate of several substances such as ethanol, nitrobenzenesulfonic acid (DNBS), rectal administration of turpentine oil, or surgical modeling by zonal suppression of blood circulation. The interval of 12-48 hours is sufficient to cause an acute inflammation, and the 30-45 days period is sufficient for developing aseptic inflammation similar to that produced by non-bacterial prostatitis [11, 12, 13, 14, 15].

Adenoprosin is a product of entomological origin, obtained by using advanced biotechnologies, which contains lipoprotein extract from Lepidopteran insect species. The product was developed and registered in 2001 within the scientific center "New Tone Laboratories" Romania. After the preclinical studies at the "Nicolae Testemitsanu" State University of Medicine and Pharmacy in the Republic of

Moldova and then in Biotehnos Bioanalysis Laboratory, Romania, it was demonstrated that Adenoprosin possesses antioxidant, antiproliferative, anti-inflammatory and immunomodulating properties.

The purpose of this study is to investigate the anti-inflammatory and adverse effects of Adenoprosin, 150 mg rectal suppositories, in experimentally-modeled aseptic prostatitis on white Wistar rats.

Material and methods

The study included 120 white Wistar male rats with an initial weight of 200 ± 2.1 grams.The acclimation period of rats to baseline was 14 days. Animals were randomized into 6 groups of 10 samples each. Each specimen was marked for identification with a specific colorant (eosin or methylene blue) according to laboratory standards. Animal maintenance and care were held according to the standards described in the "Guide for care and use of Laboratory Animals (2011, National Academy Press)" and the ISO P-53434-2009 standards of Russian Federation [16, 17].

Adenoprosin, 150 mg rectal suppositories, was administered rectally as smaller, dose-proportional parts. The dose of the product Adenoprosin was calculated as the corresponding dose recommended for human use. Thus 150mg/70kg/24 hours is equivalent to 2.1mg/kg body weight. The respective dose was adjusted for rats using the equivalence coefficient of 39/6.5, corresponding to 12.6 mg/kg [18].

The study was performed by comparing with a reference product- Vitaprost, 50 mg rectal suppositories, used for a long time for the treatment of inflammatory diseases of the prostate. The dose of the comparison product, Vitaprost, was 4.2 mg/kg.

The modelling of acute aseptic inflammation of prostate was performed in 50 rats by intra-operative ligation of the anterior lobe artery of the prostate with silk ligature. 10 rats were not subjected to surgery and were cataloged in the control group No. 1 – intact.

Four groups of 10 rats each, received Adenorposin medication for 7 days, in two different doses, or the Vitaprost comparison medication in two doses. The second dose of both products was about 3 times higher the recommended dose, i.e. 37.8 mg/kg for Adenoprosin and 12.6 mg/kg for Vitaprost.

The second control group consisted of 10 rats with acute aseptic prostatitis following placebo treatment (tab. 1).

The efficacy criteria of the treatment consisted of the: assessment of the general condition and the histological results performed 7 days after prostate ligation. Morphologically, animal necropsy was performed by determining the macroscopic appearance of the prostate, determining prostate weight, calculating mass coefficients, and performing microscopic examinations of the area of caused aseptic inflammation. Anti-inflammatory action was determined by studying the quantitative indices of acute inflammation: the surface of the epithelial cells in the stromal part of the prostate, the surface and level of blood vascularization, the number of acini with desquamated epithelium placed on 100 consecutive marginal sections [18].

Table 1

The distribution of white Wistar rats (no. 60) with acute aseptic prostatitis under study according to study groups treatment

Group No	Administered treatment	No of rats
1	Healthy rats/intact rats	10
2	Aseptic Prostatitis. Placebo	10
3	Adenoprosin – recomnended dose (12,6 mg/kg)	10
4	Adenoprosin – recommended x 3 (37,8 mg/kg)	10
5	Vitaprost – recommended dose (4,2 mg/kg)	10
6	Vitaprost – recommended dose x 3 (12,6 mg/kg)	10

The modelling of chronic aseptic prostatitis was achieved by intra-operative ligation of the right lobe of prostate gland under general anesthesia. The studied product was administered 30 days after the surgery for a period of 15 days. The distribution of the rat groups according to the administered treatment is shown in table 2.

Table 2

The distribution of white Wistar rats (no. 60) with acute aseptic prostatitis according to study groups treatment

Group No.	Administered Treatment	No of rats
1	Healthyrats/intactrats	10
2	AsepticProstatitis. Placebo	10
3	Adenoprosin – recomnended dose (12,6 mg/kg)	10
4	Adenoprosin – recommended dose x 3 (37,8 mg/kg)	10
5	Vitaprost – recommended dose (4,2 mg/kg)	10
6	Vitaprost – recommended dose x 3 (12,6 mg/kg)	10

The treatment efficacy criteria were similar to those for the study of acute inflammation, with the exception that morphological examinations were performed after 15 days of treatment, respectively 45 days after ligation of the right prostate lobe. Anti-inflammatory action was determined by studying the quantitative indications of chronic inflammation: the surface of the epithelial cells in the stromal area of the prostate, the surface and level of blood vascularization, the surface of the marginal sections of the prostate and the surface of the collagen cells in the prostate fibrous tissue layers.

The statistical processing of the data was accomplished through the statistical elaboration programs Statistica 5.5. As parametric criteria were used Student criteria and non-parametric criteria were determined according to the Wilkinson or Mann-Whitney criteria. The differences were considered true at the veracity level ($p < 0.05$).

Results

The action of the product Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories on the model of acute prostatitis

The anti-inflammatory action of the studied products on the model of acute prostatitis was appreciated. The first parameter studied was the general condition of the animals and the determination of the body mass (tab. 3).

Table 3

The dynamic of the body mas index in healthy rats and rats with aseptic acute prostatitis under the action of administered products

Assessed period	Group 1 healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	T x 3 Dose	T Dose	T x 3 Dose
Before inflammation	299,00 ±1,54	298,90 ±1,73	299,60 ±2,17	299,20 ±2,41	301,60 ±2,22	302,00 ±2,47
After 7 days	316,60 ±0,78	273,90 ±1,55*	278,60 ±1,20*	279,50 ±1,93*	280,60 ±1,77*	277,50 ±2,25*

*- statistically veridical difference compared to group 1 (healthy, intact rats), p <0.05.

From the table 3, it is clear that the acute aseptic inflammation of the prostate has had a negative impact on the rats' body mass increase in control, placebo group and the groups that were treated with Adenoprosin, rectal suppositories, or Vitaprost, rectal suppositories.

Pathomorphological studies performed 7 days after the onset of acute aseptic prostatitis determined the change in prostate weight coefficients, which are statistically veridical different (p <0.05) (tab. 4). There is, however, evidence of maintenance of the prostate weight coefficient in rats given both Adenoprosin and Vitaprost compared to the placebo group.

It was studied the microscopic appearance of the prostate in healthy rats, those given placebo, and those administered Adenoprosin or Vitaprost.

Thus, in healthy rats the parenchyma of the prostate was represented by the terminal sections of the tubular alveolar glands. Most glands had a broad lumen with a large amount of secretion, which helps the plies of the epithelial mucosa to be smooth and the epithelial cells get a cubic form. Thin layers of muscle-elastic tissue were located between the secretory units of the gland.

In animals subjected to surgical ligation of the anterior prostate lobe receiving placebo, the histological examination showed the presence of an acute prostatitis: pronounced interstitial edema with evident vascular congestion. The secretory epithelium was atrophied and absent glandular secretion.

In rats receiving Adenoprosin 150 mg rectal suppositories, the microscopic image of the prostate showed a decrease in the severity of the inflammatory process. The parenchyma of the prostate had a normal structure, the terminal sections being covered with a columnar secretory epithelium. At the same time, there were also sectors with stromal edema, but much less pronounced than in the control group without signs of vascular congestion. A similar morphological image of the prostate was observed in the animals treated with Vitaprost, 50 mg rectal suppositories (fig.1).

From a quantitative point of view, the anti-inflammatory action of the studied products was assessed by determining the surface area of epithelial cells on the gland section, the number and the surface of the blood vessels and the number of acini with desquamated epithelium per 100 consecutive marginal sections (tab. 5).

Thus, compared to intact (healthy) rats in placebo-treated animals, a significant numerical reduction of the surface of epithelial cells and blood vessels is observed, with an increased level of epithelial desquamation. In animals treated with Adenoprosin, 150 mg rectal suppositories, or with Vitaprost, 50 mg rectal suppositories, these numbers are much closer to those of the control group.

The action of the product Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories on the model of chronic prostatitis

Chronic bacterial prostatitis was modelled by intra-operative ligation of the right prostate lobe. The treatment was initiated 30 days after the ligation and had duration of 15 days. Under assessment was the action of the products on rat body mass and anti-inflammatory action confirmed by morphological changes of the prostate.

Body mass was determined every 7 days from the modelled chronic non-bacterial prostatitis and until the end of treatment. In intact animals a constant increase in body mass throughout the observation period was determined.

Table 4

Modification of the prostate weight index under the influence of the administered products

Assessed period	Group 1 Healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	T x 3 Dose	T Dose	T x 3 Dose
7 days of inflammation	2,09±0,03	1,71±0,05*	1,99±0,12#	2,12±0,10#	1,97±0,08#	2,00±0,05#

*- statistically veridical difference compared to group 1 (healthy, intact rats (p<0,05, Mann-Whitney criteria, # - veridical difference compared to control group, placebo (p<0,05).

In animals with chronic prostatitis, a progressive decrease in body mass was observed immediately after the intervention. This decrease continued in rats placebo-treated. In rats receiving the Adenoprosin or Vitaprost products, weight loss stopped immediately after initiation of therapy and after 15 days of treatment weight gain was recorded (tab. 6).

After the evaluation of the animals included in the study, was noticed that the use of Adenoprosin, 150 mg rectal suppositories, and Vitaprost 50 mg rectal suppositories, contributed to the increase in body mass index in rats with non-bacterial chronic prostatitis, compared to placebo-treated rats. Differences between the actions of the products were not noticed.

The morphological examination started with the determination of prostate weight at 45 days after the modelling of chronic prostatitis (tab. 7).

Histological examination at 45 days after modelled chronic non-bacterial prostatitis in placebo-treated rats showed the presence of interstitial edema, vascular congestion with connective tissue proliferation, high fibroblast content and inflammatory lymphocytic infiltration. The secretory epithelium was atrophied, and secretion at terminal

Table 5

The action of Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories, upon the prostate structure through the treatment of acute aseptic prostatitis (group n = 10)

Parametres	Group 1 healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	Tx 3 Dose	T Dose	T x 3 Dose
The surface of the epithelial cells	20,8±1,2	8,3±0,9*	12,4±1,3*	16±1,2#	13±1,3*	17±0,9#
The surface of the blood vessels	0,3±0,04	1,2±0,2*	0,7±0,08	0,5±0,05	0,6±0,2	0,5±0,04
The number of the acini with desquamated epithelium per 100 sections	3,2±0,09	15±1,2*	11,3±0,9*	9,4±0,7*#	11,2±0,8*	8,5±0,6*#

* - veridical difference compared to group 1, healthy, intact rats (p<0,05), Mann-Whitney criteria, # – veridical difference compared to control group, placebo (p<0,05).

Table 6

The action of Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories upon the rat's body mass index under the treatment of chronic non-bacterial prostatitis (group n =10)

Parametres	Group 1 healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	T x 3 Dose	T Dose	T x 3 Dose
Incipient	299,50±2,24	300,00 ±2,26	297,90 ± 1,57	300,30 ±1,88	296,80 ±2,00	299,70 ±2,44
7 days after	314,60 ±0,85	280,00 ±1,91*	278,60 ±1,87*	277,40 ±1,77*	282,10 ±1,96*	283,90 ±2,02*
14 days after	323,50±1,11	265,20±1,25*	265,70±0,88*	264,20±1,05*	264,00±1,24*	263,00±0,92*
21 days after	333,40±0,86	243,70±0,93*	244,00±1,04*	244,90±1,00*	244,50±1,09*	244,60±1,09*
30 days after	338,20±0,85	237,70 ±1,05*	239,10 ±1,00*	237,60 ±1,42*	237,20 ±1,00*	234,00 ±0,58*
15 days on treatment or placebo	361,60±1,16#	223,80±0,98*	259,30±1,17*#	260,10±0,99*#	260,60±0,88*#	261,60±0,86*#

* - veridical difference compared to group 1, healthy, intact rats (p<0,05), Mann-Whitney criteria, # – veridical difference compared to control group, placebo (p<0,05).

Table 7

The action of Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories upon the rats prostate weight under the treatment of chronic non-bacterial prostatitis (group n =10)

Assessed period	Group 1 healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	T x 3 Dose	T Dose	T x 3 Dose
45 days after modelling/ 15 days after treatment	2,04±0,03	1,78±0,05*	2,02±0,04#	2,00±0,03#	2,02±0,08#	2,05±0,07#

* - veridical difference compared to group 1, healthy, intact rats (p<0,05), Mann-Whitney criteria, # – veridical difference compared to control group, placebo (p<0,05).

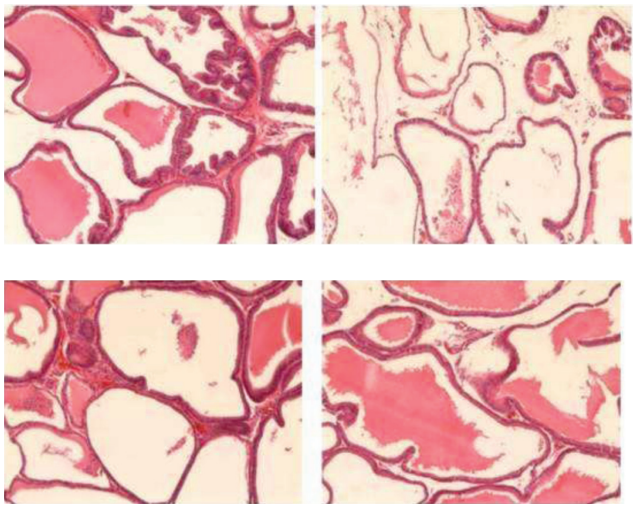

Fig. 1. Microscopic prostate structure: a) intact prostate, b) acute prostatitis treated with placebo, c) acute prostatitis treated with Adenoprosin, rectal suppositories 38 mg/kg, d) Vitaprost treated prostatitis, rectal suppositories 12 mg/kg. Hematoxylin-eosin, x 100.

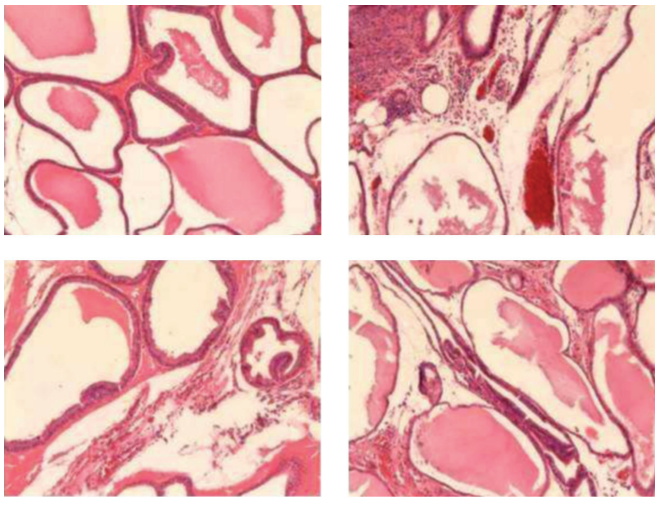

Fig. 2. Microscopic prostate structure: a) intact prostate, b) nonbacterial chronic prostatitis treated with placebo, c) non-bacterial chronic prostatitis treated with Adenoprosin, rectal suppositories 38 mg/kg, d) non-bacterial chronic prostatitis treated with Vitaprost, rectal suppositories 12 mg/kg. Hematoxylin-eosin, x 100.

Table 8

The action of Adenoprosin, 150 mg rectal suppositories, or Vitaprost, 50 mg rectal suppositories, upon the prostate structure through the treatment of chronic nonbacterial prostatitis (group n = 10)

Parametres	Group 1 healthy	Group 2 Placebo	Adenoprosin®		Vitaprost®	
			T Dose	T x 3 Dose	T Dose	T x 3 Dose
The surface of the epithelial cells	20,5±1,4	7,4±0,9*	12,3±1,2*	17,6±1,6#	11,8*±0,9	18,9±1,2#
The surface of the blood vessels	0,3±0,03	1,1±0,2*	0,6±0,2#	0,5±0,1#	0,5±0,09#	0,5±0,04#
The number of the acini with desquamated epithelium per 100 sections	3,3±0,7	50,5±1,4*	35,2±1,6*#	20,4±1,2*#	34,1±1,5*#	25,2±1,6*#
The surface of the collagen fibres	1,5±0,4	15,4±0,5*	13,6±0,7*	10,2±0,9*#	14,3±0,2*	11,1±0,5*#

* - veridical difference compared to group 1, healthy, intact rats (p<0,05), Mann-Whitney criteria, # - veridical difference compared to control group, placebo (p<0,05).

level absent. The presence of segments with epithelial desquamation and obvious dilatation of terminal sections due to exudative fluid accumulation was reported.

In rats treated with Adenoprosin, a reduction in interstitial edema and vascular congestion in the prostate was observed. The macroscopic aspect of the prostatic parenchyma was close to the usual one, secretory columnar cells and active secretion were present in the epithelium of the terminal sections.

Only a few portions have been noted with residual signs of chronic inflammation in the form of stromal lymphocytic infiltrate. The microscopic picture of the prostate of the rats treated with Vitaprost was similar (fig.2).

The anti-inflammatory effect of Adenoprosin product, 150 mg rectal suppositories, was also expressed by increasing the surface of the epithelial cells in the terminal sections, reducing the surface of vascular congestion and the number of acini with desquamated epithelium. There were no signs of an increase in the number of collagen fibers in prostate tissue (tab. 8).

In this way it has been shown that the product Adenoprosin, 150 mg rectal suppositories significantly reduce signs of inflammation in rats with modelled chronic nonbacterial prostatitis.

Discussion

The problem of chronic prostatitis is not fully elucidated. According to national and international clinical protocols the treatment of chronic prostatitis is complex with the use of antimicrobial, antiinflammatory, antioxidant, α-receptor

blockers, phytotherapeutic products, etc. The duration of treatment would be at least 28 days. At the same time, the long-term administration of some pharmacological drugs may cause a number of adverse effects, including: hypotension or retrograde ejaculation due to the use of α-blockers; libido disappearance or erectile dysfunction due to use of 5α-reductase inhibitors; peptic ulcers, dyspepsia, increased risk of cardiovascular events from using non-steroidal anti-inflammatory drugs, etc [19, 20, 21, 22].

Therefore, researches into the development of new pharmaceutical products for the treatment of chronic prostatitis continues. Evaluation of products on experimental models of prostatitis is one of the most viable solutions to appreciate their action. Most products used to treat chronic bacterial prostatitis were originally tested on animal models.

The product Cernitin, containing pollen extract, used in the treatment of chronic prostatitis has been tested on non-bacterial-induced prostatitis models in rats. Induction of non-bacterial prostatitis was modelled by administration of estradiol after castration. Histopathological evaluation of the prostate during the post-treatment period determined amelioration of the epithelial score, respectively the reduction of the glandular atrophy, along with the inhibition of stromal proliferation [23].

Another herbal product (WSY-1075), tested on non-bacterial prostatitis models induced in Wistar rats, showed that the product significantly reduced the level of prostate proinflammatory cytokines (IL-6 and IL-8) and histological lesions after 8 weeks of administration compared to the control group [24].

A Finnish study has shown that the spruce extract, orally administered for 18 weeks to rats with non-bacterial prostatitis induced experimentally relieves pain and urine evacuation without adverse effects [25].

Several studies recommend the use of products with organotropic action on the prostate for the improvement of local haemodynamic indices [26, 27, 28].

Prostatilen, a product similar to the product included in our study, Vitaprost, contains regulating peptides extracted from the bovine prostate, was tested in rat models weighing 180-200g, to which prostatitis was induced by injection of 10% dimexide solution. The experiment demonstrated the organotropic anti-inflammatory effect of the product [29].

The use of bovine prostate peptides (cytomedins) has been known for many years. They are recommended for their local anti-inflammatory effect and immunomodulatory properties. In several clinical trials, their effect on the evolution of chronic prostatitis and benign prostatic hyperplasia was noted [30, 31].

Adenoprosin is a product that contains lipoproteins that have organotropic action on the prostate. These lipoproteins are obtained from Lymantria dispar larvae by a major new method. Biologically active components of the product contribute to the reduction of A2 phospholipase formation and arachidonic acid elimination, followed by a decrease in prostaglandin and leukotriene production [32].

The study assessed the effect of Adenoprosin on the experimental model of non-bacterial acute and chronic prostatitis administered as rectal suppositories. After the morphological examination of the aseptic acute prostatitis model, a decrease in the severity of the inflammatory process, manifested by decreased stromal edema and vascular congestion, was observed. After the quantitative evaluation of Adenoprosin action compared to the control group treated with placebo and the reference product Vitaprost, in animals without organotropic treatment it was observed a significantly reduced surface of epithelial cells, reduced number of blood vessels, and increased level of epithelial desquamation. In rats with aseptic acute prostatitis treated with Adenoprosin or Vitaprost, these indices were much closer to the control group and healthy rats.

On the experimental model of non-bacterial chronic prostatitis, both the tested and the reference products acted by reducing interstitial edema and vascular congestion. The histological appearance of the prostate is suggestive. In rats with non-bacterial chronic prostatitis without treatment, the secretory epithelium is clearly atrophied, interstitial edema and vascular congestion are reported. Under the action of organotropic products, there is a significant reduction in edema, and residual signs of inflammation are present only in certain sectors.

The results of our study are encouraging and open new perspectives for the treatment of non-bacterial chronic prostatitis.

Conclusions

The product of entomological origin Adenoprosin 150 mg rectal suppositories tested in the experimental study of aseptic acute prostatitis showed an obvious anti-inflammatory effect.

The anti-inflammatory activity of the product Adenoprosin is also manifested in the non-bacterial chronic prostatitis model by restoring morphological status of the secretory epithelium, reducing interstitial edema, vascular congestion and the number of acini with prostate desquamation.

The anti-inflammatory activity of Adenoprosin 150 mg rectal suppository on the experimental model of aseptic or chronic non-bacterial prostatitis induced on Wistar white rats is similar to that of the reference product Vitaprost, 50 mg rectal suppositories and is significant compared to placebo.

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Variants of branching of the upper limb arteries

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Abstract

Background: The study of anatomical variability is a component of one of the largest compartments of anatomy and it is a current direction of the modern morphology, conditioned by the nowadays requirements of practical medicine. The presence of anatomical variants is closely related to the abnormal development of the arterial system during intrauterine life, mainly that of the primary axial artery of the upper limb. Those variants do not lead to functional disorders, but they may become fatal under certain circumstances.

Material and methods: During the dissection of a 60-year-old male cadaver an unusual arterial variant was found out on the right upper limb, using classical methods of the upper limb arteries dissection.

Results: In its retropectoral part the axillary artery was bifurcated into two arterial trunks, the brachioradial and brachioulnar arteries. The brachioradial artery represented the anterior trunk of the axillary artery bifurcation, having a superficial trajectory, while the brachioulnar artery was the posterior and deeply located one. In the specialty references, the brahioradial and brachioulnar arteries are defined as high origin of radial and ulnar arteries, which arise more common from the brachial artery and less frequently from the axillary one.

Conclusions: The variants of origin and trajectory of the upper limb arteries are of clinical significance to both imagists and vascular surgeons. The imagists may misinterpret the angiographic images with such vascular patterns and surgeons may encounter difficulties in surgery at that level.

Key words: axillary artery, brahioradial artery, brachioulnar artery.

Introduction

The specialty references describe the variability of the upper limb arteries as one of the most common variations and it is reported by different researches. According to McCormack L. the upper limb arterial variants were depicted in 18.5% of cases, and according to Uglietta J. – the incidence of variations was 9%, and among those the most common was related to the axillary artery, followed by the radial and brachial arteries [1, 2, 3].

Unlike anatomical variants of the venous system, most of the arterial ones, do not affect the functions of the human body, being detected during dissection, or when performing angiography at that level [4].

The appearance of different variants of the upper limb arteries may be caused by genetic factors, or by the disturbances of the development of the primary axial artery, which is related to the embryonic period, dependent of oxygenation and nutrition, and related to the hemodynamic force of the blood system, but at the same time, it might be influenced by the local factors, such as fetal position in the uterus, early limb movements, or unusual muscular development [5, 6].

Malci-Gurbuz I. [7] sustains that genetic factors are the most probable cause leading to arterial variations during angiogenesis; and as a result of that complex and dynamic process, it is not surprising that the variability of the axillary and brachial arteries is quite common.

Arey L. B. [8] mentioned that arterial variations appear as consequences of unusual way of development of the primary vascular plexus, or because of the presence and development of the blood vessels that under normal conditions should go through involution. In some cases, arterial variants are formed due to the regression and disappearance of blood vessels that under normal conditions must be present, or due to the incomplete development of the blood vessels, their fusion or resorption of some of them, which normally do exist separately.

The topographic and numerical variability of the upper limb arteries is of clinical significance for imagistic diagnostics, and important for surgeons in choosing the right way and tactics in stenting coronary angioplasty [9].

Material and methods

At the Department of Human Anatomy, of Nicolae Testemitsanu State University of Medicine and Pharmacy, during the dissection of a 60-year-old male cadaver an unusual arterial variant was found out on the right upper limb. When preparing the axillary artery, its bifurcation into two arterial trunks at the retropectoral level – the brahioradial artery and brachioulnar artery, was observed (fig.1).

Before its bifurcation, the axillary artery was of 5 cm in length and its external diameter was 0.5 cm. In its first portion the axillary artery had a usual trajectory and it had given off its typical branches: the superior thoracic and thoracoacromial arteries.

The brachioradial artery was the anterior trunk of the axillary artery bifurcation, while the brachioulnar artery –

Fig. 1. Branching of the axillary artery: 1 – axillary artery; 2 – bifurcation of the axillary artery; 3 – brachioulnar artery; 4 – brachioradial artery.

Fig. 2. Arteries of the forearm: 1 – brachioradial artery; 2 – brachioulnar artery; 3 – superficial palmar branch.

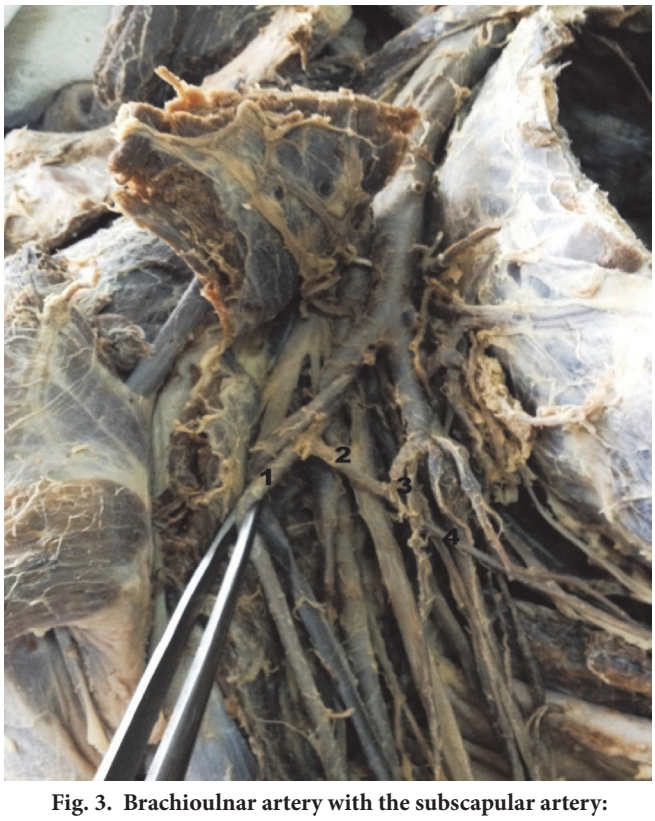

Fig. 3. Brachioulnar artery with the subscapular artery: 1 – brachioulnar artery; 2 – subscapular artery; 3 – circumflex scapular artery; 4 – thoracodorsal artery.

Fig. 4. Brachioulnar artery and its branches: 1 – brachioulnar artery; 2 – common arterial trunk; 3 – posterior circumflex humeral artery; 4 – anterior circumflex humeral artery; 5 – deep brachial artery; 6 – radial nerve.

the posterior trunk; the last one had a larger external diameter (which proximally was 0.5 cm and distally – 0.3 cm), in comparison with the external diameter (proximally it was 0.3 cm and distally – 0.24 cm) of the brachioradial artery.

On the arm the brachioradial artery was located along the medial bicipital groove, and in its upper third the artery passed medially to the median nerve and brachial veins; in the middle part of the arm it crossed them anteriorly, to change its position, so, in the lower third of the arm – the brachioradial artery had the most lateral position.

In the cubital fossa it passed behind the aponeurosis of the biceps brachii muscle to continue on the forearm with the trajectory characteristic of the radial artery (fig. 2).

At the level of the pectoral triangle, the lateral thoracic artery originated from the brachioradial artery, while on the arm it did not give off any branches, only on the forearm it gave off the recurrent radial artery, muscular branches and the palmar superficial branch.

At the level of the subpectoral triangle, the brachioulnar artery was located posteriorly and deeper than the brachioradial one, then it continued on the arm along the medial bicipital groove, being located between the brachial veins and laterally to the median nerve, while on the forearm it had a common for the ulnar artery trajectory, giving off branches characteristic of it.

In the subpectoral triangle, the brachioulnar artery gave rise to the subscapular artery with the external diameter of 0.2 cm, and it divided into its thoracodorsal and circumflex scapular arteries (fig. 3).

At the lower margin of the pectoralis major muscle, from the brachioulnar artery originated a common trunk of the same diameter as the subscapular artery, that immediately branched out into three arteries: the anterior circumflex humeral artery with a diameter of 0.03 cm, that emerged from the brachioulnar artery; the posterior circumflex humeral artery with a diameter of 0.1 cm and the deep brachial one – 0.08 cm in diameter (fig. 4).

At the level of the upper third of the arm, the brachioulnar artery gave off the superior collateral ulnar artery and in the inferior third – the inferior collateral ulnar artery; the external diameter of the first one was 0.04 cm, and its angle of origin was 55°, the diameter of the second one was 0.02 cm, and the angle of its origin – 80°; the upper collateral ulnar artery bifurcated into two branches: a muscular one, which supplied the medial head of the triceps brachii muscle and an articular branch – to the elbow joint; the inferior collateral ulnar artery was a single arterial trunk, that participated in formation of the elbow joint arterial network.

On the forearm, the brachioulnar artery had the usual to the ulnar artery trajectory, lodging in the ulnar groove and giving off its typical branches.

Discussion

The brachioradial artery is defined as the high origin of the radial artery that may be present on the arm with the brachial artery, or with superficial brachial one, at the level, where those arteries branch out into the ulnar and common interosseous arteries [4].

The brachioradial artery in the specialty reference sources is described as the most common arterial variant of the upper limb, originating from the axillary, or from the brachial artery (table 1).

According to F. Fuss [10] the brachioradial artery, is more frequently present in males and predominantly on the right side, though these differences are not so significant, that fact was also confirmed by A. Rodriguez-Baeza [11].

From the topographic point of view, the brachioradial artery crosses the median nerve anterosuperiorly on the entire length of the arm, while the brachial artery – posteriorly or in some cases – anteriorly; therefore the brachioradial artery was named by some researchers – as superficial brachial artery [12, 4].

According to our case, in the cubital fossa, the brachioradial artery passed behind the bicipital aponeurosis and it was also confirmed by some authors, based on case study references [13].

The brachioradial artery can anastomose with the brachial artery by means of a spiral, or by a straight branch [11, 5].

Table 1

Origin of the brachioradial artery according to the reference sources based on a large research

Author	Number of cases	Axillary artery	Brachial artery	Brachial artery		
				Upper 1/3 of the arm	Middle 1/3 of the arm	Lower 1/3 of the arm
Quain R. (1844)	53	16 (30%)	37 (70%)	19(35.9%)	13 (24.6%)	5 (9.5%)
Muller E. (1903)	31	8 (25.8%)	23 (74.2%)	22 (71%)	1 (3.3%)	0
Adachi B. (1928)	29	9 (31%)	20 (69%)	-	-	-
Karlsson S. (1982)	8	1 (12.5%)	7 (87.5%)	5 (62.5%)	2 (25%)	0
Uglietta J. (1989)	8	1 (12.5%)	7 (87.5%)	2 (25%)	2 (25%)	0
Rodrıguez-Baeza A. (1995)	6	1 (16.7%)	5 (83.3%)	3 (50%)	1 (16.65%)	1 (16.65%)
Rodriguez-Niedenfuhr M. (2001)	52	12 (23%)	40 (77%)	34 (65.4%)	4 (7.7%)	2 (3.9%)
Vandana N. (2012)	20	6 (8.3%)	14 (19.6%)	-	-	-
Chandni Gupta, (2012)	12	2 (2.66%)	10 (26.6%)	-	-	-

The presence of the brachioradial, or of the median artery is a common variation due to their high incidence, while the origin of a median artery from the brachioradial one, in the distal third of the forearm mentioned by Muler [14] is a rare variant; the occurrence of that variant can be explained by close correlation between the radial and medial arteries and by the anastomosis between them at the level of the forearm during embryogenesis [4].

The brachioulnar artery is defined as a high origin of the ulnar artery, which may exist as an arterial variant of the upper limb, along with the brachial artery, that bifurcates into the radial and common interosseous arteries.

That pattern of axillary artery branching is less frequent than the presence of the brachioradial artery, and in terms of its distribution by gender and body part, the specialty literature does not make any references, since it has almost always been occasionally detected [15,16].

In the specialty references, a high origin of the ulnar artery was most commonly mentioned, when it started in the upper third of the arm, but it originated very rarely from the retropectoral portion of the axillary artery [17, 18].

Uglietta J. [2] noted that in 2% of cases, the ulnar artery had its origin from the axillary artery, having a monolateral character, and according to Al-Sowayigh M. [19] the incidence was 1.7%.

According to the bibliographic data, at the level of the arm, the brachioulnar artery passes in front of the median nerve, and on the forearm – beneath the bicipital aponeurosis, and then continues the trajectory common to the ulnar artery [20]. This fact was confirmed by the data of our study.

Rodriguez-Niedenfuhr M. [4] performed the study on 384 upper limb samples and he analyzed statistically the distribution of the anatomical variants by gender and body part. The similar data were obtained in our case. Table 2 shows the data obtained by Rodriguez-Niedenfuhr M.

Table 2

Distribution of the brachioradial and brachioulnar arteries by gender and part of the body (M. Rodriguez-Niedenfuhr)

Gender	Number	Brachioradial artery		Brachioulnar artery	
		Left	Right	Left	Right
Male	91	9 (9.9%)	11 (12.1%)	3 (3.3%)	3 (3.3%)
Female	101	14 (13.9%)	19 (18.8%)	5 (4.9%)	5 (4.9%)
Total	192	23 (12%)	30 (15.6%)	8 (4.2%)	8 (4.2%)

Conclusions

The variants of origin and trajectory of the upper limb arteries are of clinical significance to both imagists and vascular surgeons. The imagists may misinterpret the angiographic images with such vascular patterns and surgeons may encounter difficulties in surgery at that level.

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Oxidative stress and inflammation biomarkers in pulmonary tuberculosis

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Abstract

Background: Tuberculosis outcome and clinical features of the infection are influenced by the degree of the multiplication of mycobacterias, host's defense mechanisms and the organism's capacity to fight through the antioxidant mechanisms against the aggression of the oxidative stress. The aim of the study was to assess the oxidative stress and inflammatory biomarkers in pulmonary tuberculosis.
Material and methods: A prospective study, which included 46 patients with pulmonary tuberculosis and 36 healthy persons determined according to the clinical and biochemical criteria, was performed. The oxidative stress was assessed through the level of the advanced oxidation protein products, advanced glycation end-products, fibrinogen, amino acid catabolic products, activity of N-acetyl-β-D-glucosaminidase. The determination of the total antioxidant activity of plasma was performed through ABTS and CUPRAC methods. IL-8 and TNF-α were assessed using analysis kits of BOSTER (USA) producer.
Results: Was established high level of the oxidative stress following the assessment of the concentration of the advanced oxidation protein products, advanced glycation end-products, fibrinogen, N-acetyl-β-D-glucosaminidase, urea and creatinine. High concentration of amino acid catabolic products was attributed to the nephrotoxic properties of the medication. Was identified high level of the plasma total antioxidant activity and antioxidant compounds. Cytokines concentration IL-8 and TNF-α was several times higher than in the control group and they were assessed as specific biomarkers.
Conclusions: High level of the protein peroxidation, advanced glycation end-products, fibrinogen, protein catabolism compounds, pro-inflammatory cytokines – IL-8 and TNF-α confirmed the boosting of the oxidative stress. The elevated total antioxidant activity and antioxidant proteins demonstrated the organism's capacity to redress the oxidative aggression.

Key words: tuberculosis, oxidative stress, IL-8, TNF-α.

Introduction

Tuberculosis (TB) represents a multifactorial disease with an evolution and treatment response determined by the continuous interaction between *Mycobacterial tuberculosis* (MBT) and human genotype. Natural history and morphological features of the tuberculous infection are influenced by the degree of the multiplication and dissemination of MBT and host's defense mechanisms [5]. The resistance against micobacterial infection is performed by macrophages through phagocitosis of MBT and by CD4 lymphocytes through interleukin production. If at least 5 MBT achieve the lung of a previously non-infected host there are two possibilities of outcome: 1) alveolar macrophages destroy MBT through their phagocytosis, 10% of cases; 2) surviving and intracellular division of the MBT [1]. The host response against mycobacterial infection consists of two phases: a 3rd type hypersensitivity reaction induced by the immune circulating complexes (antibody mediated) which is developing in 2-3 weeks after the infection and a 4th type hypersensitivity (cell-mediated) developed in 8-12 weeks. The 3rd type immune response is morphopathologically characterized by exudative lesions rich in active MBT and the 4th type represents nodular-proliferative granulomas which contain the latent forms of MBT [1].

The nonspecific resistance of the organism against infection, including TB is based on the recognizing of the specific antigens as well as of the common antigenic groups called pathogen associated molecular patterns (PAMPs) [1]. The typical PAMPs are constituted from different substances such as: lipopolisaharides, endotoxins, peptidoglicans, viral nucleic acids, fungus β-glicans, flagelines, lipoteichoic acid, etc. The recognizing of the PAMPs is realised by the specific membrane receptors, defined as pattern-recognition receptors (PRR). The most important representatives of the PRR implicated in the TB immunity are: Toll-like receptors (TLRs), C-type lecithin receptors (CLRs) and Nod-like receptors (NLRs) [1]. The specified receptors are a family of the transmembranes proteins identified in many immune cells (macrophages, neutrophyles, dendritic cells, lymphocytes, mastocytes) and non-immune cells (enterocytes, astrocytes, hepatocytes, epithelial cells, etc.). Their stimulation will activate the gene response by the production and releasing of the different types of the immune inductors: interleukins (IL), interferons (IFN), hematopoetic growth factors, tumor necrosis factors (TNF) and chemokines [1, 5, 9,]. Interleukins regulate the systemic inflammatory response before the development of the adaptive immunity. Pro-inflammatory cytokines are IL-1, IL-6, IL-8, IL-12 and are secreted as a specific response to specific molecules PAMPs that bind to pattern recognition receptors (PRRs), including Toll-like receptors (TLRs) [1]. IL-1 is a mitogenic protein, a lymphocyte B activating factor and a lymphocyte B differentiation factor [1]. IL-2 are secreted by the lectin stimulated T lymphocytes and B lymphocytes. It induces the differentiation of the T lymphocytes and activates B lymphocytes as a growth factor, being the antibody secretion

stimulant [1]. IL-6 is secreted by the T lymphocytes and macrophages during infection, inflammation, trauma as a specific response to PAMPs. It mediates the acute phase response, the production of neutrophils and the maturation of B lymphocytes. IL-6 is the major regulator of the lymphocytes B transformation into plasmocytes [1]. IL-8 is released by phagocytes and mesenchymal cells induced by the IL-1 and TNF-α. It activates neutrophil chemotaxis and their accumulation, lysosomal exocytosis and the OS at the site of the infection, inflammation, tissues ischemia or traumatism [4, 8, 9]. IL-12 is produced by the macrophages, neutrophils, dendritic cells, B-lymphoblastoid cells as a response to antigenic stimulation. The main function represents the differentiation of T lymphocytes into T helper 1 lymphocytes. It stimulates the production of IFN-γ and TNF-α from lymphocytes T and natural killer cells. TNF superfamily is a family of cytokines that cause the cell apoptosis [14]. The first described was TNF- α (also named cachectin) known as monocyte-derived cytotoxin involved in the cytolysis of certain cell lines, induces cachexia, fever (by IL-1 secretion) and cell differentiation [5]. The clinical expression of the cytokines is various, but the most of them cause the endogenous intoxication syndrome. Besides the immune response, the disease outcome depends on the organism's capacity to fight through the antioxidant mechanisms against the aggression of the oxidative stress (OS) determined by the released mycobacterial exotoxins and antituberculosis drugs [8]. OS is caused by the imbalance between the production of the free oxygen radicals and the capacity of the biological system to detoxify the peroxides and free radicals [12]. OS is manifested through the peroxidation of the cellular DNA, proteins, lipids, carbohydrates and other biological macromolecules. OS and protein peroxidation determine chronic metabolic disturbances with extensive fibrosis and collagen accumulation in the tissues, in consequence developing the multisystemic organ failure. The advanced oxidation protein products (AOPP) are important biomarkers of the OS. Those are constituted from uremic toxins which result from the interaction between the chlorine oxidants (chloramines and hypochlorus acid) with plasmatic proteins. The kidneys, spleen and the liver are major organs responsible for the isolation and excretion of the AOPP [16]. The increased blood concentration of AOPP is established in chronic inflammatory systemic diseases (systemic sclerosis), chronic kidney disease, hyperparathyroidism, atherosclerosis, diabetes mellitus, and during the treatment with calcium and vitamin D [16]. AOPP are structurally similar to advanced glycation end-products (AGEs) [15]. Elevated blood concentration of the AGEs can indicate the glucide metabolism disorders. The highest concentration of the AGEs is identified in diabetes mellitus patients and is determined by the non enzymatic glycosilation of the proteins and excessive activation of the polyol way during the hyperglycemia. The AGEs are heterogenous substances which result from the non-enzymatic glycation of the proteins, lipids and nucleic acid during a chain of reaction, defined Maillard reaction [11, 15]. Fibrinogen is an acute phase protein and rises in response to systemic inflammation, infections, trauma, cancer and thrombosis. Fibronogen is the biomarker of OS and inflammation, that demonstrates the functional effects on the fibrin clotting [1]. The antioxidant system is composed by the hydrophilic antioxidant compounds identified in the cytoplasm, blood serum and by the hydrophobic molecules localized in the biological membranes. Enzymatic antioxidants from the blood and cellular cytoplasm are: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPO) and gluthatione-S-transferase (GST) [1].

The N-acetyl-beta-D-glucosaminidase (NAG) is a high molecular-weight hydrolytic lysosomal enzyme identified in different tissues (liver, kidneys, lungs, lymph, tears, urine, blood, etc.). It hydrolyses chemical chains of glycosides and amino carbohydrates that form structural components of the cell membrane and lysosomal membrane [17]. In the lungs this enzyme is secreted by alveolar macrophages in response to phagocytosis. Its role in the control of infection consists in the sterilizing activity within the intracellular compartment of the macrophages [17]. Deficiency of the enzyme determines the susceptibility for reactivation of latent TB infection, dissemination and poor treatment outcome. High activity of NAG in the bronchoalveolar lavage indicates acute or chronic pulmonary injury, fibrosis, exposure to fibrogenic and nonfibrogenic dusts. The highest concentration of NAG is established in the proximal tubular cells of the kidneys. High activity in the urine provides information about the impairment of the tubular functions resulting from a disease, nephrotoxicity of the anti-TB drugs and associated OS [17]. The anti-TB treatment is an important risk factor for the metabolic disorders and elevation of OS [12]. The treatment for the drug susceptible TB consists in an association of the 1st line antituberculosis drugs (isoniazid, rifampicin, pyrazinamide, ethambutol and/or streptomycin) during 6 months and for the drug-resistant TB an association of the 2nd line antituberculosis drugs for 18-24 months. Adverse drug reactions are important clinical signs of the OS. The most frequent anti-TB drug reactions associated with the OS are the hepatotoxicity and nephrotoxicity [12]. This study reflects a comparison of the OS indices, antioxidative activity and some inflammatory biomarkers in patients with pulmonary tuberculosis during the intensive phase of the treatment compared with a representative sample of healthy persons. The aim of the study was the assessment of the oxidative stress, antioxidative activity and inflammatory biomarkers in pulmonary TB.

Material and methods

It was realised a prospective study which included 46 patients with pulmonary tuberculosis (study group) diagnosed in the municipal specialized institutions of Chisinau during the period 01.01.2016-31.08.2016 and 36 healthy persons determined according to the clinical and biochemical criteria (control group). Including criteria in the study

group were: age more than 18 years old, patient diagnosed with pulmonary TB, patient type "new case", the diagnosis confirmed through the conventional microbiological methods, patients treated in the intensive phase in the frame of the Municipal Hospital of Phtisiopneumology in Chisinau and signed informed consent. The study schedule included information about the sex, age, clinical radiological diagnosis, case type, patient's microbiological status and results of the drug susceptibility test, treatment regimen and adverse drug reactions. All patients included in the study were treated according to the national clinical protocol "Tuberculosis at adults". The including criteria in the control group were: age more than 18 years old, healthy individual according to the clinical criteria and laboratory findings (complete blood count, biochemical tests; liver transaminases, blood electrolytes and signed informed consent. The assessment of the immune biochemical indices in the serum was performed using the methods with microquantities of the blood serum and work reagents. The dosage was performed in micro plates with 96 wells, but the filling with the samples and reagents was performed with the automatic multichannel pipettes. The measure was performed using the chemical reagents and assessing the absorbance with the spectrophotometer in the maximum standardization of conditions. The total proteins were assessed according to the modified Lowry method [7]. The OS was assessed through the determination of the AOPP according to the modified method of Witko-Sarsat V. [7, 16] and AGEs according to the modified method of Sero L. [7, 13]. It included the spectrophotometric measure of the two main types of the AGEs: *pentosidine-like* and *vesperlysines-like.* The micromethod was based on the fluorescence measure of the intensity of the studied samples diluted in the phosphate tampon at λexc 335 nm, λem 385 nm (quuantification of the *pentosidine-like* AGEs) and at λexc 370 nm, λem 440 nm (quantification of the *vesperlysines-like* AGEs) [7, 11]. The concentration of the urea and serum creatinine was assessed through the spectrophotometric analysis using the kits of the producer Eliteh (France) according to the attached instructions [6]. The concentration of fibrinogen was assessed using the kits of the producer Eliteh (France) according to the attached instructions [6].

The determination of the plasma total antioxidant activity was performed through two procedures: method based on the degradation of the 2,2-azino bis (3-ethylbenzotiazoline-6-sulphonic acid (ABTS) radical at the interaction with serum compounds with the antioxidant properties and measure of the decreasing absorbance at 734 nm [7] and CUPRAC method (*Cupric Ion Reducing Antioxidant Capacity*) based on the reducing capacity of the Cu ion through the capture of the hydroxyl radical [2, 7]. The activity of N-acetyl-β-D-glucosaminidase was assessed according to the Gudumac V. method [6]. The concentration of the immune cytokines of the IL-8 and TNF-α was assessed using the ELISA kits of the producer BOSTER (USA) according to the attached instructions.

The study methodology was based on the collection, statistical analysis, graphic representation and analytical assessment. Statistical analysis was realized by comparative assessment of the quantitative and qualitative features of the selected patients using the Microsoft Excel XP programme. Accumulated material was systematized in simple and complex groups. For the assessment of differences between the indices of the compared samples it was performed the statistic non-parametric test "t test" and the significance threshold "p" ($p<0,05$).

Results

Distributing patients, according to the biological characteristics, a similar rate of men and women was set in both groups, with the predomination of men in the same proportion in both groups which ensured the comparability of the results. The same proportion of young persons aged less than 44 years was established in both groups, which was accepted as a condition permitting the comparability of the laboratory data (fig. 1).

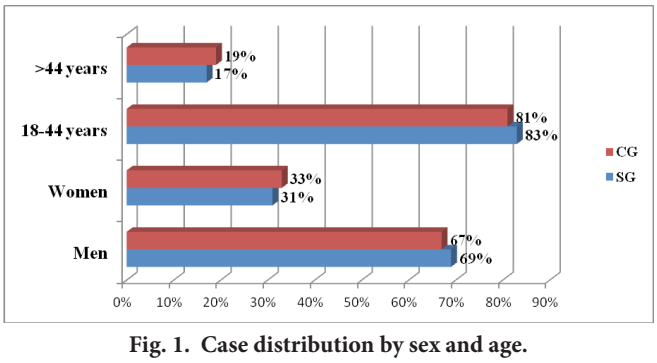

Fig. 1. Case distribution by sex and age.

Detected by passive way were 28 (56.52%) patients in the frame of the symptomatic case examination, 7 (15.21%) through the examination of high risk groups and 16 (34.78%) by direct addressing to the specialized health institution. The majority of patients, 43 (95.65%), were diagnosed with pulmonary infiltrative tuberculosis and 3 (4.35%) with disseminated tuberculosis (fig. 2). At the radiological examination was identified lung destruction in all patients of the study group. Microscopic examination of the smear for acid-fast-bacilli was positive in 30 (65.22%) cases. The conventional cultures revealed MBT colonies in 26 (56.52%) cases. The drug susceptibility test established 20 (43.47%) drug susceptible and 6 (13.04%) drug resistant strains of MBT. Monoresistance to isoniazid was established in 2 (4.34%) cases, but the polyresistance to isoniazid and streptomycin in 3 (6.52%) cases.

Standardized treatment for drug-sensitive TB was administrated in 31 (67.39%) patients, standardized treatment for MDR-TB (DOTS-Plus) in 13 (28.26%) patients and individualised regimen for polyresistant tuberculosis in 2 (4.34%) patients. Immune biochemical indices were analysed at 46 patients with pulmonary tuberculosis (study group) during the intensive phase of the treatment in the hospital performed according to the drug susceptibility test.

The collection (harvesting) of the blood of the control group was performed in ambulatory conditions.

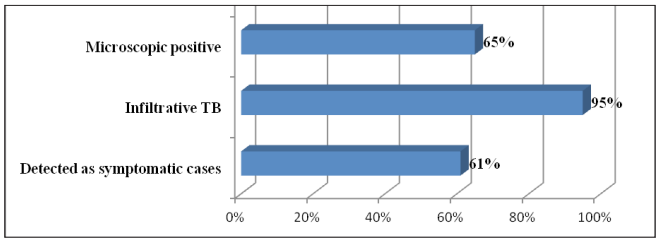

Fig. 2. Clinical, radiological and microbiological characteristics of the tuberculosis patients.

Fibrinogen concentration, as a biomarker of the OS was more elevated in the group of the patients with tuberculosis compared to the control group. During the assessment of the most important products of the amino acid catabolism, was established that the concentration of urea in blood was significantly higher in the group of the patients with tuberculosis compared to the control group. In the same way were established the disturbances of the creatinine concentration, which was significantlty higher in the group of patients with tuberculosis (tab. 1).

Assessing obtained data it was established a statistical higher concentration of the AOPP in the group of patients with tuberculosis compared to the control group. The serum concentration of the AGEs *pentosidine-like* assessed through the fluorescence at 330 ex/390 em established a nonsignificant lower concentration in the group of patients with tuberculosis compared to the control group, but the concentration of the AGEs *vesperlysines-like* at the fluorescence at 370 ex/440 em demonstrated a significant higher level in the group of the patients with tuberculosis compared to the control group (fig. 3).

Assessing the results of the antioxidant activity of the serum through the method CUPRAC and ABTS it was established a significant increasing of the antioxidant system compounds in the group of patients with tuberculosis compared to the control group. The concentration of the ceruloplasmine, known as an acute phase protein with antioxidant role, was significantly higher in the group of patients with tuberculosis as well. The concentration of the total serum proteins (albumin and globulin α1, α2, β, γ) with antioxidant role was established in a higher concentration in the group of patients with tuberculosis (fig 4).

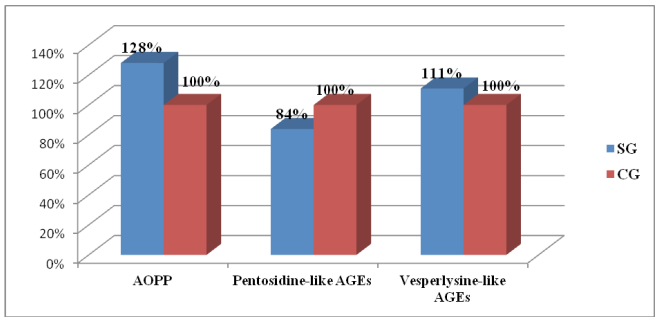

Fig. 3. Oxidative stress biomarkers in the blood.

Note: AOPP – advanced oxidation protein products, AGEs – advanced glycation end-products (AGEs).

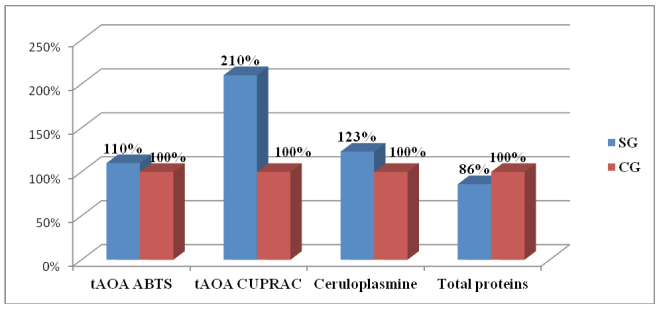

Fig. 4 Comparative assessment of the antioxidant ativity and antioxidant compounds of the blood serum.

Table 1

Some indices of the oxidative stress

Oxidative system	Parameter	SG (N=46)	CG (N=36)	P
		M±SE	M±SE	
Oxidative stress biomarkers	AOPP μMol/l	44,06±2,86 (128%)	34,349±3,58 (100%)	**0,032**
	Pentosidine-like AGEs μg/ml	174,3±15,41 (84%)	208,5±16,27 (100%)	0,13
	Vesperlysines-like AGEs, μg/ml	382,2±25,42 (111%)	343,2±49,63 (100%)	0,45
	Fibrinogen mg/dl	40,90±1,02 (182%)	22,45±0,7 (100%)	**<0,0001**
Amino acid catabolism products	Urea mg/dL	18,92±9,2 (145%)	13,00±2,28 (100%)	**0,02**
	Creatinine mg/dL	79,79±6,84 (173%)	45,87±5,69 (100%)	**0,0003**

Note: AOPP – advanced oxidation protein products, AGEs – advanced glycation end-products (AGEs).

Table 2

Some indices of the antioxidative activity

Antioxidant defense	Parameter	SG (N=46)	CG (N=36)	P
		M±SE	M±SE	
Total antioxidant activity (tAOA)	Method ABTS mMol/l	0,77±0,005 (110%)	0,71±0,004 (100%)	**<0,0001**
	Method CUPRAC mMol/l	1,09±0,18 (210%)	0,517±0,04 (100%)	**0,008**
Proteins with antioxidant role	Ceruloplasmine mg/l	887,2±36,48 (123%)	724,3±27,8 (100%)	**0,0008**
	Serum total protein g/l	59,4±3,61 (114%)	57,1±2,3 (100%)	**0,001**

In terms of the quantitative data the total antioxidant activity determination was more elevated being assessed through the CUPRAC method compared with ABTS. Ceruloplasmine, an acute phase reactant with copper-dependent anti-oxidant activity, was more elevated than the serum total proteins, which include albumin and globulins (α1, α2, β and γ globulins). Data are shown in the table 2.

Assessed inflammatory biomarkers constituted the activity of the N-acetyl-β-D-glucosaminidase (NAG), the concentration of IL-8 and TNF-α. The NAG activity was higher in the group of patients with tuberculosis in comparison with the control group. The concentration of the pro-inflammatory cytokine IL-8 was 10 times higher in the serum of the patients with tuberculosis in comparison with the control group. The concentration of the TNF-α was three times higher than in the control group (tab. 3).

Table 3

Pro-inflammatory biomarkers

Parameters	SG (N=46)	CG (N=36)	P
	M±SE	M±SE	
NAG	80,48±5,315 (122%)	65,88±3,06 (100%)	**0,027**
IL-8 ng/ml	15,595±8,411 (1134,05%)	1,163±1,685 (100%)	**<0,0001**
TNF-α pg/ml	212,41±195,5 (323%)	65,78±12,09 (100%)	<0,0001

NG N-acetyl-β-D-glucosaminidase.

Discussion

Distribution of patients in sex and age groups determined the predomination of the men and economic reproductive age in both selected samples, which allowed the comparability of the results. Diagnosed through at least one microbiological conventional method the patients with a wrong diagnosis were excluded. The diagnosis of the pulmonary infiltrative tuberculosis and lung destruction in a high propotion demonstrated the similarity of the selected group with the national cohorts [10].

Estimation of the level of the oxidative stress markers through the serum concentration of the advanced oxidation protein products, fibrinogen and products of the protein catabolism demonstrated the presence of a higher peroxidative stress in the group of patients with tuberculosis. Studies evaluating advanced oxidation protein products and advanced glycation end-products in patients with tuberculosis in the specialised literature have not been identified. High values of urea and creatinine are comparable to the results of international studies, being attributed to the nephrotoxic properties of medications of the aminoglycoside group included in the regimen of every patient, but also can be explained by the exacerbation of the catabolism [8].

The concentration of the *pentosidine-like* advanced glycation end-products at a lower level in patients with tuberculosis, demonstrated the metabolic changes during starvation. The concentration of the *vesperlysine-like* advanced glycation end-products was insignificantly higher in the group of tuberculosis patients. The level was lower than in comorbid diabetic patients associated with tuberculosis reported in international studies [11].

The markers of the serum antioxidant system underwent elevated changes in the group of the tuberculosis patients. The similar results from the specialised literature demonstrated the hyperactivity of the defensive mechanisms against mycobacterial exotoxins as well as the increased metabolic detoxification of the antituberculosis medication [8]. The intensification of the blood antioxidant activity in patients with pulmonary tuberculosis was demonstrated also by the high concentration of the ceruloplasmine and total serum proteins. The same results were identified in other scientific papers [3].

The elevated activity of NAG in the blood of patients with pulmonary tuberculosis indicated pulmonary injury due to infectious aggresion of MBT. No similar studies were reported in the specialized literature. The quantitative immunoassay revealed that IL-8 was significantly elevated in the patients with tuberculosis. Considering the fact that IL-8 is a chamotactic factor for neutrophils, lymphocytes T and basophils its pivotal role in the modulation of acute and chronic inflammation can be deducted by obtained results also [4, 9]. Assessment of TNF-α established it as one of the most important mediator of the inflammation in the antimycobacterial cytokine cascade that suggests acute pulmonary inflammation and apoptosis in pulmonary tuberculosis [14].

Conclusions

In conclusion, in tuberculosis the increased level of the protein peroxidation, advanced glycation end products, fibrinogen, protein catabolism compounds and pro-inflammatory cytokines: IL-8 and TNF-α confirmed the boosting of the oxidative stress. The increased total antioxidant activity, elevated concentration of the proteins with the antioxidant role in pulmonary tuberculosis demonstrated the organism's capacity to redress the oxidative aggression.

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REVIEW ARTICLES

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Peculiarities of using drugs in the elderly

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Abstract

Background: The global aging of the population is particularly evident in economically developed countries and has a progressive character, being in the sight of the state, government and of many international organizations. According to statistics, in this period of life, the morbidity is higher in males than females. At the same time, the need to provide medical assistance to the elderly is 50 percent higher than needed for middle-aged people. About 26% of senile patients show complications and side effects due to medications. The reasons may vary: the late doctor attendance, the socio-psychological state, polymorbidity, the chronic outbreaks of diseases, parallel treatment at other physicians', self-treatment etc. The simultaneous treatment by other physicians, who in their turn, prescribe drugs which might increase the probability of chemical and physical incompatibilities, and especially the pharmacological ones. Self-treatment is a global issue. The patient, quite often, listens to the advice of neighbors, friends, acquaintances who believe they are suffering from the same disease. So appear the different forms of polypragmasia.

Conclusions: So appear the different forms of polypragmasia. Polypragmasia is sometimes more dangerous than the insufficient treatment. Anatomical-physiological modifications of the cardiovascular system of senile patients lead to paradox effects of the administration of some drugs. Before initiating any treatment, it is necessary to determine whether the patient is using any drugs recommended by other specialists, friends, neighbors or drugs, which are not allowed to be prescribed with other drugs, in order to avoid unwanted interactions.

Key words: elderly, polymorbidity, polypragmasia, self-treatment.

Introduction

The global aging of the population is particularly evident in economically developed countries and has a progressive character, being in the sight of the state, government and of many international organizations [51].

If in 1950 the 60-year-olds would make 200 million, then in 2025 it is foreseen for them to rise 6 times, as many as 1.2 billion people [37].

Nowadays, the old patient presents a unique clinical-psychological phenomenon in terms of presence and association of pathologies according to character and manifestation, due to the involution changes of different organs and systems [9,31,51].

In the structure of the diseases of senile patients pathologies of the cardiovascular system (ischemic cardiopathy, arterial hypertension, atherosclerosis) are found most frequently; diseases of the CNS and sensory organs rank second; more rarely – diabetes mellitus, ophthalmologic diseases, vascular pathologies, gastro-intestinal diseases etc [16,19]. According to statistics, in this period of life, the morbidity is higher in males than females. At the same time, the need to provide medical assistance to the elderly is 50 percent higher than needed for middle-aged people [2,28].

Since the major role in geriatric medicine is given to the medicament therapy, the physician must constantly perfect his ability of rational tactics, to assure maximal results and minimal risks of developing complications. According to the latest data, this group of people develop adverse reactions two to three times more often than younger groups of people [32,33,35,37].

Starting the treatment, it has to be taken into consideration that patients of higher age usually suffer from three to four, ten or even twelve chronic diseases simultaneously. In this situation, there is a necessity to take several different drugs at once. Therefore, the pharmacotherapy for this category of patients demands a strict evidence of all possible medicament interactions, since both, the risk of relative overdose and the risk of side effects rise [6,14].

The presence of psychosomatic disorders contributes to the worsening of the pathogenesis, influencing the forecast and the quality of life. They make it hard to identify the correct diagnosis and to choose the most fitting method of treatment, to select the proper dose of medicament [3,13].

The treatment, including that with medicaments, is a major step that represents a permanent problem in the geriatric practice – "to treat or not to treat, how and with what?"

Younger people have more stable homeostatic mechanisms to keep the organism healthy than older people. That is why aging is characterized by the diminution of these adaptive processes [17].

About 26% of senile patients show complications and side effects due to medications. The reasons may vary: the late doctor attendance, the socio-psychological state, polymorbidity, the chronic outbreaks of diseases, parallel treatment at other physicians', self-treatment etc [4,5,12,27].

One of the important factors is polymorbidity. The administration of medications for the treatment of a disease may lead to the worsening of another one or to the development of complications. For example, a prescription with high potential in treating pulmonary diseases may provoke vomiting due to its mechanism, leading to lesions of vessels and external and internal hemorrhages. These complications require effort and expenses for the stabilization of the patient [22,34].

Chronic disease outbreaks demand the usage of medications for a long time. That is why before initiating treatment it is necessary to collect a precise "drug history"- what, for how long and what dosage was prescribed to the patient. At the same time, we need to take into consideration that not all new medications have passed the required clinical trial for this category of patients. Administering them, the doctor runs the risk of registering adverse reactions in the patient [7,11,20].

The atypical process of pathologies, and their difficult diagnosis, many times contributes to the usage of symptomatic treatment, which is often not completely effective. As a consequence of the disease's progress, saving the patient in the late stages of the disease might require major doses, which therefore increase the risk of adverse reactions even more [26,30].

The doctor should take into account a possible simultaneous treatment by other physicians, who in their turn prescribe drugs, which might increase the probability of chemical and physical incompatibilities, and especially the pharmacological ones [18,20].

Self-treatment is a global issue. The patient, quite often, listens to the advice of neighbors, friends, acquaintances who believe they are suffering from the same disease. This is how different forms of polypragmasia appear. Polypragmasia is sometimes more dangerous than the insufficient treatment. It has been determined that a simultaneous use of 6 or more medical remedies in older patients, is the reason that 80% of the cases develop unfavorable adverse reactions. Combining drugs cannot only increase the potency of the necessary pharmacological effect, but also the toxic one, which can manifest itself by increased weakness, fatigue, dizziness, sleep problems, movement issues [8,15,19]. However, doctors often attribute these symptoms to age, without thinking that they might constitute a relative overdose of drug usage.

It should be taken into account that not only the factors enumerated above can provoke adverse reactions, but also the particularities of geriatric pharmacokinetics – the path of the drugs from administration to the elimination from the organism (tab. 1).

Owing to saliva insufficiency in the buccal cavity, the medication processing is disturbed and there is a decreased ability of fermentation, as well. Under these conditions, the drug arrives in the stomach in a dry state [38].

Loss of 20% of mucosal surface may be caused by retention of absorption. This functional remodeling of the digestive tract leads to the retention of absorption of the drug followed by delay of appearance of the therapeutic effect. On the other hand, the constipation present in most of the cases, by which intestinal hypomotor is manifested, can increase the bioavailability of the drugs [41,44].

The decrease in number of capillaries and their increase of winding in patients over 60 lead to the reduction of drug absorption when administered subcutaneously or intramuscularly. Therefore, these ways of administration have to be avoided in these patients, especially oily forms [24,40].

The pharmacologic and toxic effect of the drug mostly depends on its distribution in the organism. Taking into consideration the fact that a person close to the age of 80 loses 10-15% of the circulating liquid, it leads to the decrease of microcirculation. The drug, arriving in a small volume of liquid, increases in concentration and creates an overdose. For example, after one hour, the concentration of propranolol in blood may be up to 4 times higher in older people than in younger ones [28,39,50].

The disturbance of distribution of the drug within the senile organism depends on the physico-chemical modification of the blood, the permeability of the tissues and the connection with the plasma proteins, especially with

Table 1

Peculiarities of physiological changes in older patients

Process	Character of modification	Consequences
Absorption	Reduced: formation of hydrochloric acid in the stomach, discharge velocity, TGI motility, circulation in mesenteries vessels, absorption area. The microscopic study of intestinal biopsy in the elderly demonstrated 20% decrease in mucosal surface, skin layer atrophy, reduction of capillary numbers and increase of their winding with reduction in blood circulation.	It increases the latency of the effect, increases the duration of action, more often hypoxia, intoxication
Distribution	Cell dehydration, reduced muscle tissue mass and increased fatty mass, tissue perfusion, atrophy or decreased parenchymal organ mass.	Relative overdose
Binding with plasma proteins	Decrease of albumin-concentration in blood plasma.	Increase in effectiveness of drug, often side effects
Metabolism	Reduced: liver mass, hepatic circulation, fermentation activity participating in the metabolism of the drug and contributing to the accumulation of toxic intermediate products.	Increase of duration of drug action
Excretion	Reduced degree of glomerular filtration and canaliculi secretion	Increase of duration of drug action leading to overdose

the background of hypoalbuminemia. hypoalbuminemia causes the increase of the free fraction of the drug and the formation of a toxic effect [41,49].

In senile patients the intensity of metabolic reactions of the drug in the liver decreases, followed by accumulation of intermediate substances which is toxic for the organism. At the same time, growth of adipose tissue of the liver, in which the drug deposits, creates a higher probability of manifestation of the toxic effect [19,43,44,45].

Reduction of the cortical layer by 20%, reduction of the speed of blood flow in kidneys by two times, reduction of the glomerular filtration rate by three times may retain the drug in the patient's organism. As digoxin has a half-life of 51 hours in patients of 40 years, yet in 70-year-olds it has a half-life of around 73 hours. Also, gentamicin has a half-life of 1.6 hours in 40-year-olds but of 5.6 hours in 70-year-olds [23,30,33].

Anatomical-physiological modifications of the cardiovascular system of senile patients lead to paradox effects of the administration of some drugs as papaverines, nitroglycerines. They may increase arterial pressure in older people, yet in other situations they may provoke a colaptoid effect. Barbiturates may lead to irritability; caffeine may have a sedative effect [1]. Based on the peculiarities described above, it is necessary to follow certain principles of drug administration in older patients.

Before initiating any treatment, it is necessary to determine whether the patient is using any drugs recommended by other specialists, friends, neighbors or drugs, which are not allowed to be prescribed with other drugs, in order to avoid unwanted interactions.

The dosage of the drug has to be ½ or 2/3 of the dosage for young adults taking the involution peculiarities of the organism into consideration. For senile patients, the body mass is not a criterion to determine the dosage of a drug.

To prevent polypragmasis, basic pathologies have to be determined and treated [20,49].

To avoid overdose (due to inability of the patient to break the pill in half, etc.) the way of drug administration must be easy. The dosage of the prescribed drug must be obeyed.

To prevent doubling or tripling of dosage because of memory loss of the patient, it is recommended to part the drugs in boxes for "morning", "afternoon", "evening", etc.

In order to avoid chemical interactions, the drugs must be administered with an interval of 30 minutes minimum.

To avoid ulceration of the digestive tract mucosa in older patients, acidic drugs (non-steroidal anti-inflammatory, sulfanilamide, analgesics) should be taken after meals [35,50].

To avoid physicochemical interactions, the composition of meals should be taken into consideration when administering drugs. For example, caffeine-containing tetracycline and other caffeine-containing drugs require milk and dairy products to be eliminated from the diet, in contrary when administering NSAIDs and glucocorticoids it is better to include dairy products. [7, 47, 48].

Some drugs, as iron, papaverine, atropine, are not recommended to be used together with tea or juices because these contain tannin, which deregulates the absorption of the drugs. It is also recommended to avoid fruit and vegetable juice when taking erythromycin, ampicillin and grapefruit juice when taking calcium, yet grapefruit and orange juice enhance the absorption and effect of hypnotics. Antibiotics and drugs against tuberculosis require many fruits and vegetables [29, 39].

Fatty food products are not recommended to be ingested with acetylsalicylic acid, furadonine, nitroxoline or sulfanilamide, because it decreases their absorption. However, fats are well suited for the administration of anticoagulants, vitamins A, D, E, K, diazepam and aminophylline, as fats increase the absorption of these preparations.

Protein-rich meals contribute to the increase in blood proteins, which bind to the drug to decrease the amount of free drug and the pharmacologic effect of the following preparations: cardiac glycosides, anticoagulants, sulfanilamide, quinidine, theophylline, caffeine, cimetidine, etc. Some drugs disturb the proteic, glucidic and lipidic metabolisms. For preventing these complications, proteins (cheese, fish and meat), potassium (apples, peaches, beans, carrots, peas, onions, etc.), calcium (dairy products) and vitamins are recommended [51].

Analgesics require the exclusion of smoked food. To elude complications, for senile patients administration of several analgesics simultaneously and for more than ten days should be avoided. Sometimes, the toxicity of a drug is associated with alcohol. Examples are the usage of paracetamol or acetylsalicylic acid with alcohol, which leads to an increase of the hepatotoxic and nephrotoxic effects of these drugs.

Ways of reducing unwanted effects have to be taken into consideration, too (tab. 2).

Table 2

Ways of reducing unwanted effects

Drug	Schedule of administration
Clorpromazine, Clonidine, Metildopa, Hidralazine	To lie down for 1.5 / 2 hours after administration
Piracetam,, Diuretics, Decamevit, Expectorants	Not to be administered at night
Etacrinic acid	To be administered after breakfast
Diclofenac, Dipiridamol, Complamin	To be swallow without chewing
Nicotinic acid, Nicospan	To be administered 10 minutes after meals

Last but not least, the biological rhythm, both individual and common, are taken into account for a more effective and bearable action. The importance of the biological rhythm has been demonstrated for several groups of drugs: glucocorticoids, methylxanthines, antihypertensives, etc. The action of antihistamines is of a longer duration if given at 07:00 in the morning. The best time for acetylsalicylic acid administration as an antiplatelet is 08:00 in the morning because that is when the gastroduodenal mucosa is less sensitive and therefore less vulnerable. NSAIDs are recommended between 08:00 and 12:00 o`clock. Antihypertensive medications are recommended to be given 1.5 – 2 hours before nictemeral blood pressure elevation peaks to increase

the duration of the effect of the hypotensive drug. The latter is due to the functional cumulative effect, which allows reducing the number of administrations, the cost of treatment and unwanted side effects [42, 43].

Another important factor is the socio-psychological state of the senile patient. The physician should use principles for protecting the psyche of the patient by using rational psychotherapy- explaining clearly about the disease, the possible ways of treatment, their advantages and disadvantages. Sadly, physicians often ignore the importance of this component, faulting the patient's decreased hearing and memory for the low efficiency of the treatment.

Conclusions

All this, following a self-analysis, may lead to neurotic, depressive and phobic disorders which often complicate treatment, making it ineffective. It has been demonstrated that depressive patients undergo treatment less effectively than active patients [10,25]. Therefore, it is very important for a physician to gain authority and trust in front of the patient. Markers that are necessary for the prophylaxis of a curative heterogeneity are:

- Agreement between patient and physician about the need of treatment
- Simple indications about the schedule of administration in terms of number of drugs, number of administrations and side effects
- Active implication of relatives, social workers, pharmacists, etc.

When administering drugs to senile patients the physician is always obliged to ask himself about the vital necessity of this drug to this patient at this specific time [18,21,52].

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From body mass index to body composition analysis in diagnostic of childhood obesity

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Abstract

Background: The prevalence of obesity increased worldwide in children and adolescents from 1975 to 2016. The recent increase in childhood obesity has led to an interest in the question of which definitions should be used to distinguish the obese child. Body mass index (BMI) was recommended for use in children to assess body weight status. There are several international (World Health Organization, International Obesity Task Force, Center for Disease Control) and national BMI cut-offs references, and it is a major obstacle in studying global secular trends for younger age groups. Moreover, BMI does not distinguish between increased mass in the form of fat, lean tissue or bone, and hence can lead to significant misclassification. The ideal monitoring tool should directly assess adiposity. All available body composition methods in children are indirect. The gold standard for body composition is the four compartment (4-C) model. Although bioelectrical impedance analysis is most susceptible to imprecision when compared with the 4-C model it is the most logical bedside method to apply in children owing to its low cost, noninvasiveness, lack of radiation exposure and ease of use.

Conclusions: BMI may produce a significant level of misclassification. Population-based cut-off values for body fat determined by body composition reference methods are the best criterion. Bioelectrical impedance is inexpensive, portable, simple and rapid to use. Further studies to elucidate the relationship among BMI, body fatness, fat distribution, and health risks in children should be followed.

Key words: body mass index, children, obesity, body composition, bioelectrical impedance analysis.

Introduction

Obesity is the most important nutritional-health problem of children and teenagers in developed countries [1]. Because of urbanization, life style change, and modernization, mean body mass index (BMI) and prevalence of obesity increased worldwide in children and adolescents from 1975 to 2016 [2]. According to the World Health Organization (WHO) report, more than 1.9 billion of adults (39%), 41 million children under 5, and 340 million children aged 5-19 have already been overweight in 2016 [1]. However, if post-2000 trends continue, child and adolescent obesity is expected to surpass moderate and severe underweight by 2022 [1].

The WHO defines obesity as an excess in fat mass great enough to increase the risk of morbidity, altered physical, psychological, or social well-being and/or mortality [2]. Obese children are more likely to become obese adults, and the biological changes that lead to obesity-related cardio-metabolic disease start to develop in childhood. In addition, hypertension, left ventricle hypertrophy, and high serum lipids have already been described in children with obesity. Other obesity related disorders such as T2DM, depression, sleep disorders, and asthma have been observed in children as well [27]. Obesity early in life is considered to be a risk factor for death from cardiovascular disease and from all causes in adulthood; such obesity may limit the increase in life expectancy that otherwise would be achieved. Despite progress in prevention and treatment of cardiovascular disease, cardiovascular mortality among young adults either has not declined or the decline has slowed over recent decades in several developed countries coincident with the obesity epidemic [9,10 29].

BMI for definition of obesity in children. There is now considerable concern over the trend towards increasing fatness in children, and in the recent marked increase in childhood obesity. These trends have led to an interest in the question of which definitions should be used to distinguish the obese child, and whether the same definitions are appropriate for clinical practice and epidemiology [3].

An ideal measure of body fatness should meet several requirements: it should be accurate in assessing the amount of body fat; it needs to be precise with small measurement error; the measure can predict risks of health consequences; it should be possible to develop cut-offs to separate individuals according to their adiposity-related health risks and it needs to be feasible in terms of simplicity, cost and ease of use, and acceptability to the subjects [4]. Although none of the existing measures satisfies all these criteria, the current consensus is that BMI is probably the best choice among available measures [3]. BMI calculated as weight in kilograms divided by the square of height in meters (kg/m2), is a measure of weight adjusted for height, as the scaling of body weight to height across adults provides powers rounded to 2 [5]. It was first described in the 19th century by a Belgian mathematician who noticed that in people he considered to be 'normal frame', the weight was proportional to the height squared [6]. Actual BMI has been recommended for use in children, adolescents, and adults to assess body weight status [3, 4], but whereas in adults the BMI cut points that

define obesity and overweight are not linked to age and do not differ for males and females, in growing children BMI varies with age and sex [5,7].

Growth curves giving BMI distribution as a function of age and sex have then been elaborated so as to ensure more adapted application of this tool in the pediatric population [2]. The curves currently available were developed in response to the need for appropriate evaluation of body weight status and obesity in children on a national level (e.g. France, Germany, Great Britain, India, China) and/or internationally (e.g. WHO International Obesity Task Force, IOTF) [2, 7]. There are widely used three classification systems for ages 5 to 18 years which were developed with different objectives [8].

In the early 1980s the first BMI charts were published [11]. Following publication of French BMI references, Must's references generated from data gathered in the National Health and Nutrition Examination Survey I (NHANES I) in the USA were published in 1991, and their use was recommended by the WHO in 1995 [12]. Subsequently, other references from various countries were published. In 2000 in the USA, the Centers for Disease Control (CDC) and Prevention published sex-specific BMI-for-age growth charts [13]. Generally, references were based on nationally representative data, without selection criteria for feeding practices. The new WHO standards, released in 2006 for assessing the growth of children from birth to five years of age, were constructed differently [14]. They were created from samples made up of healthy breast-fed children from various countries around the world, and were intended to present a 'standard 'of physiological growth rather than a descriptive "reference" [8]. In order to extend these growth curves to school age children and adolescence, in 2007 the WHO developed references for 5- to 19-year-olds based on data from US surveys [15, 11]. In 2000, the International Obesity Task Force (IOTF) developed BMI centiles constructed on the basis of 6 nationally representative data sets to define childhood overweight and obesity [16]. As for the CDC [13] and WHO references [14], the same data from US surveys were incorporated in the IOTF references, leading to some similarities between reference curves (tab.1) [11, 21].

The US BMI-for-age reference is based on nationally representative data from boys and girls ages 2–20 years collected between 1963 and 1980 [2]. National reference standards are also in use in the UK, and are under development elsewhere [7]. Controversy exists about whether and under what circumstances a national or international reference standard is best [7]. The IOTF references have several advantages: they are internationally based and, because they are built to pass through adult cut-offs which are linked with mortality rates, they are less arbitrary than other cut-offs; they are also less geographically and temporally dependent than some other references [11]. WHO standards and references also have several advantages: they display data from birth and references for various anthropometric measurements. In addition, the WHO software converts anthropometric measurements into SDS allowing to express measurements as continuous variables and to define high levels of excess weight [11].

Moreover, the fact that the comparison with the IOTF and the CDC and the WHO reference give different prevalence estimates proves that these references represent populations between them. Perhaps in this case, it will be more appropriate to develop and use a local reference, in particular for BMI for age, where body distribution of fat might be more genetically determined [8]. Thus, the plethora of references that can be used makes it difficult to choose between them and to have a clear idea of childhood obesity prevalence worldwide [11]. The methodological problem of inconsistency between criteria of childhood obesity classification is a major obstacle in studying global secular trends for younger age groups [17].

Table 1

Common classifications of Body Weight (adults and children)

Classifications of BW (adults and children)	Age	Indicator	Normal Weight	Overweight	Obese
Adults	≥20 years	BMI (kg/m2)	18.50 to 24.99	≥25.00 Preobesec: 25.00 to 29.99	≥30.00 Class 1: 30.00 to 34.99 Class 2: 35.00 to 39.99 Class 3: ≥40.00
Children					
WHO 2006	0-60 months	BMI Z or WH Z	>−2 to ≤2 SD risk of overweight:>1 to ≤2 SD	>2 to ≤3 SD	>3 SD
WHO 2007	5-19 years	BMI Z	>−2 to ≤1 SD	>1 to ≤2 SD	>2 SD
IOTF	2-18 years	Growth curve for BMI at age 18		BMI = 25	BMI = 30
USA	2-19 years	BMI percentile	≥5th to <85th	≥85th to <95th	

Abbreviations used: BMI, body mass index; IOTF, International Obesity Task Force; SD, standard deviation; WHO, World Health Organization; WH weight-for-height; Z, z score.

Limitations of BMI. Although it is common to use BMI for determining obesity, where only height and weight are needed to be measured, it is not an accurate criterion for obesity evaluation [3,16,18,22,27]. Weight can be divided into two components: FM (fat mass) + FFM (fat free mass). FFM is a complex tissue compartment composed of skeletal muscle, organs, bone, and supporting tissue [30]. The FFM and FM indices are equivalent concepts to the BMI (as the denominator is the same), and result from the partitioning of BMI into two subcomponents using body composition, namely, BMI kg/m2= FFMI kg/m2 + FMI kg/m2; hence FFMI = (BMI - FMI) and FMI = (BMI- FFM) [28,35].

Thus, FFMI and FMI use similar ratios for their calculation as does BMI, the only difference being that the numerator is composed of FFM or FM rather than body weight also in kg. Considering the equation above, an increase (or a decrease) in BMI could be accounted for by an increase (or a decrease) in either subcomponents (FFMI or FMI) or in both components [28]. BMI does not distinguish between increased mass in the form of fat (FM), lean tissue or bone (FFM), and hence can lead to significant misclassification [3,6,18,27,28]. For example, body builders and competition athletes in other power and strength sports (boxing, shot put, wrestling and culturism) have a low proportion of fat in the body, but their BMI is often in the overweight/obese range because of their large lean (muscle) mass [28]. On the basis of their BMI, normal individuals may carry a high percentage of body fat [19], almost during puberty. Although weight gain is also a result of increased muscle mass and adipose tissue in both sexes during puberty [9], the gain in muscle mass is higher in boys and that for adipose tissue is higher in girls and normal children by BMI/age, may carry excess body fat and are metabolically similar to those carrying excess weight [18,19]. Thus, unlike in adults where BMI is generally uncorrelated with stature, some studies show that BMI and stature are related in children, particularly during early adolescence in boys, so, children and younger adolescents, particularly boys, who are tall for their ages may have large BMI values as a consequence of stature rather than excess adiposity [20,31,32]. The BMI alone cannot determine the nutritional status of overweight or obese adolescents, limiting its exclusive use [19,27]. Sidhu *et al.* analyzed sensitivity, specificity, and accuracy of BMI in determining high body fat mass by conducting a study on 500 girls within the age range of 6- to 11-year old. In this study, the BMI of equal to and more than the 95th percentile of CDC standards was regarded as obesity and the fat mass (measured by caliper) of equal to or more than the 90th percentile was regarded as having high fat mass. This study reported sensitivity, specificity, and accuracy as 42%, 85%, and 87%, respectively. Also, it concluded that using BMI by itself was not appropriate for determining high fat mass and suggested using another indicator along with BMI for determining obesity and high body fat mass [22].

The fact that body mass index represents only a crude proxy for body fat and may produce a significant level of misclassification is universally accepted but widely ignored. Another study was focused on the ability of BMI with 85th to 94th percentiles to identify children with high body fat correctly. Among children who had a BMI for age between the 85th and 94th percentiles, about one-half of these children had a moderate level of fatness, but 30% had a normal fatness and 20% had an elevated fatness [5]. A study comparing Asian prepubertal children from New York City (NYC) and Jinan, Shandong, mainland China stated that although no differences were found in mean BMI, Jinan Asians had significantly higher percent body fat (%BF) compared with the NYC Asians (P<0.001), being both samples collected from urban settings on two separate continents [17]. Low-moderate sensitivity as a marker of adiposity is a problem for public health applications such as surveillance of obesity, because large numbers of children with excess body fat will not be identified [7].

Body composition reference methods. Since the pathology associated with obesity is driven by the excess fat mass, the ideal monitoring tool should directly assess adiposity [18]. Population-based cut-off values for body fat determined by body composition reference methods are theoretically the best criterion for the definition of overweight and obesity [2]. It should be emphasized, however, that body fat is measured with a much greater error than body weight and height. Consequently, this would explain why any potential superiority of body composition measurements over BMI in predicting health risks is difficult to demonstrate [28]. Still, over the past several decades body composition methods have been gaining acceptance in both research and clinical medicine [4,39]. Many studies have demonstrated that adult body composition measurement methods and data may not be directly applicable to pediatric populations [5,6,9].

Despite the fact that numerous techniques are now available for estimating body composition, there is no single method for measurements in vivo [30]. All methods incorporate assumptions that do not apply in all individuals, and the more accurate models are derived by a combination of measurements, thereby reducing the importance of each assumption [28].

All approaches to body composition analysis can be organized according to the number of compartments described. *Two-compartment models* (2-C) divide the body into fat mass (FM) and fat free mass (FFM) such that total body mass = FM + FFM [30]. The direct measurement of body fat mass has never been easy and remains a significant challenge for most body composition techniques. However, if one can determine the total FFM, then body fat can be defined indirectly as the difference between body weight and FFM. The 2-C model, which has been used in body composition research for more than 50 years, continues to serve a vital role, especially in the evaluation of newer technologies focusing on body fat assessment [35]. Two compartment methods include anthropometry, densitometry, bioelectric impedance, or isotope dilution for total body

water. *Three-compartment (3-C) models* divide body mass further into FM, non-osseous lean body mass (LBM) and bone mass such that total body mass = FM + LBM + bone mass. In this 3-C model, the FFM is divided into two parts: its water content and the remaining solids (predominately protein and minerals) [35]. For this 3-C model, the density of water, fat, and body solids are used. The results obtained using this model provided some improvement over the basic 2-C model for healthy adults and older children. However, for patients with significantly depleted body protein mass and/or bone mineral mass, the estimated values for the density for the solids compartment would be incorrect; thus the final estimate of body fat mass was also inaccurate [35]. DXA offers a quick, convenient means of three compartment analysis. Because DXA measures bone mineral content directly, this method eliminates one of the major sources of variability inherent in the estimation of the FFM in the two-compartment model [30, 37]. To extend the basic 2-C model to *four compartments (4-C)*, one would need an accurate measure of the protein and mineral compartments, in addition to that of total body water. For this four-component model, the densities for body protein and bone mineral can be assumed as 1.34 and 3.075 kg/l, respectively. Multicomponent models using methods or combinations of methods to measure FM + three or more components of FFM have also been developed. The accuracy of body composition assessment improves with the number of components measured as there is less dependency on the assumption that FFM density is constant [30]. For example, the formula for a 4-C model might include density values for fat, water, mineral and protein [36]. However, the 4-C model is generally not available to clinicians, because of the need for specialized equipment. Although other methods, such as quantitative computed tomography, magnetic resonance imaging and magnetic resonance spectroscopy, are used to determine the quantity and quality of adipose tissue, skeletal muscle and other internal tissues and organs, they have limited usefulness for the clinician, because they are not necessarily available for nondiagnostic use, are expensive and require highly specialized equipment and technicians and may expose children to radiation (for example, neutron activation, computerized tomography scan) [4,36]. Thus, the clinician must primarily rely on techniques that are based on the two-compartment model for routine determination of body composition in children, including dual-energy X-ray absorptiometry (DXA), dilution techniques, hydrodensitometry (also known as underwater weighing) and air displacement plethysmography, single- and multi-frequency bioelectrical impedance analyses (BIA).

Dual-energy X-ray absorptiometry (DXA) devices estimate FM% with acceptable accuracy and have become the reference method for estimating body composition [2]. DXA is a widely recognized method of body composition analysis that beyond BMD provides information of the nutritional status of the patients, including fat reserve and lean soft tissue [26]. However, their drawbacks are radiation exposure, relatively high cost, and limited accessibility. Bioelectrical impedance techniques are typically developed and validated against DXA, dilution and/or hydrodensitometry techniques, which serve as reference methods for that purpose [6,10].Compared to DXA, bio impedance analysis (BIA) has been shown to provide a good degree of accuracy in various populations [21].

Although BIA was the technique most susceptible to imprecision when compared with the 4-C model [12], it is the most logical bedside method to apply in children owing to its low cost, noninvasiveness, lack of radiation exposure and ease of use and better reproducibility compared with other bedside techniques, such as skinfold measurements [4,18].

The bioimpedance (BIA) method is based on the concept that tissues rich in water and electrolytes conduct better the flow of an electrical current than adipose tissue. Bioimpedance systems measure the impedance of a low energy electrical signal as it flows through body tissues; impedance is proportional to the conductor length (i.e., height) and inversely proportional to the conductor crossectional area. Four electrodes are usually attached to the individual during measurement: from hand to hand and from foot to foot with the subject standing. Conduction of the electrical current through body tissues is related to the water and electrolyte content of the tissue [4,6]. It is important to note that measurement conditions are fundamental for obtaining accurate BIA body composition estimates. The BIA model, the equation used for body composition estimation, room and subject temperature, body position, electrode placement and several other factors (e.g., eating or drinking, dehydration, exercise) can all influence measurements and should be standardized during measurement. The subject must be lying horizontal at least 5 min or more before measurement, to allow an even distribution of all the body fluids. In presence of fever BIA data are not valid. Since the volume to be assessed is the entire length between the foot and the arm it is important to avoid any contact that short circuits such pathway. If the subject is not dressed, arms and legs must be separated from each other, or insulated. To avoid acute fluid shifts, subjects will be instructed to refrain from strenuous exercise for 12 h before the measurement. The examination must be done after an overnight fasting. Room temperature must be kept between 20 and 24°C to prevent undesired effects on cutaneous blood flow or compartmental changes in water. Subjects will be measured while lying supine on a non-conductive surface [4].

Percent body fat (fat mass(kg)/body mass(kg)* 100) is obtained from body composition methods that estimates fat mass and provides more valuable information than BMI by differentiating between fat and fat free mass. A study comparing BMI to percent body fat (% FM) found that less than half of children and adolescents defined as overweight by BMI (BMI ≥ 85th percentile) had high adiposity defined by percent body fat [30]. Flegal M et al. showed that current BMI cut-offs can identify a high prevalence of high adiposity in children with high BMI-for-age and a low prevalence

of high adiposity in children with normal BMI-for-age [33].

Nowadays, there is no consensus about %FM cut-offs for obesity in children and adolescents. Especially during adolescence, the level of adiposity may vary widely by age, sex and pubertal development [4]. Normal patterns of body fat include a decrease in body fat percentage after infancy and subsequent increase in body fat percentage until puberty. In normal growth and development in children, males gain more muscle and lean tissue than fat at puberty while girls gain more fat [6]. The reference values and chart created with selected percentiles of the normal adolescents might be helpful in growth assessment and obesity related risk evaluation [5]. The national percentile values of %FM, according to age and gender, were identified in many countries for evaluating distribution of body composition in adolescents [5,34]. In the absence of clear cut-off points, usually accepted %FM values for the definition of excess body fat range between 30–35% in female adolescents and 20–25% in males aged 4–6 years and 15–18 years [4].

The use of percent body fat (%FM) is limited by the fact that it does not take into account the effects of height, body proportion, and the independent contributions of absolute amounts of fat and fat free mass to health and disease [30]. Fat mass index (FMI) is obtained from dividing body fat mass (kg) by squared height (m^2) can be a proper criterion for predicting body fat mass and obesity. It provides the possibility for considering body fat mass separately and stating it relative to height [23]; it is used in some studies for determining obesity as a better criterion than body fat percent [22]. FMI was found to be more sensitive indicators of nutrition status compared to BMI or percent body fat when applied to data from the Minnesota Semi-Starvation Study. Analyses of FMI and FFMI (fat free mass index) in children have revealed that increases in BMI during childhood are largely driven by increases in FFMI and not FMI, suggesting that BMI may not accurately represent adiposity in all situations [32].

By determining these indices, quantification of the amount of excess (or deficit) FFM and FM can be calculated for each individual. Thus, the calculation of FFMI will allow a clinician to identify a malnourished individual, whereas interpretation of BMI and FM% may fail to detect the presence of protein–energy malnutrition [38]. Although BMI is a useful tool to compare body weights in individuals who differ in height, FFMI and FMI are useful for the comparison of body composition in individuals who differ in height. The advantage of the combined use of these indexes is that one can judge whether the deficit or excess of body weight is selectively due to a change in FFM, FM or both combined. For example, an individual of 1.85 m and 100 kg, and hence having a BMI of 29.2 kg m2, would be judged as largely overweight and even borderline obese. This would be true if his FMI is higher than the reference values and conversely if his FFMI is not simultaneously elevated. Another advantage of FMI, as compared with the BMI concept, is that it amplifies the relative effect of aging on body fat. Expression of a change in relative body FM (%) alone fails to allow an appropriate comparison among subjects of different sizes. The high sensitivity of FMI (or conversely of FFMI) to a slight change in body fat stores (or conversely lean tissue mass), compared with the use of BMI or FM% as factors, makes it an index of potential interest for assessing static and dynamic nutritional status and energy reserve end points. The concept of FFMI could also be useful for calculating the relative muscle hypertrophy in bodybuilding and other sports, in which heavy muscular body build needs to be measured quantitatively to exclude false diagnosis of excess body fat based on single BMI measurements [28].

No reference values have been specified for FMI yet. The use of this index, which is promising but requires a valid assessment of body composition by the pediatrician, is increasingly under evaluation [28]. Reference intervals of FMI versus FFMI, for adults, children and teenagers, can be used as indicative values for the evaluation of nutritional status (degree of overnutrition or undernutrition) of apparently healthy subjects. It can also provide complementary information to the classical expression of body composition reference values. FMI is able to identify individuals with elevated BMI but without excess FM. Conversely, FMI can identify subjects with 'normal' BMI but who are at potential risk because of elevated FM [28]. It was observed that 79% of the obese children based on FMI were recognized to be obese based on BMI as well and 73% of the children with normal adiposity based on FMI showed the same status with BMI; in other words, sensitivity and specificity of BMI in comparison with those of FMI as the real criterion of obesity were 79% and 73%, respectively [22]. Based on these results, BMI compared with FMI as the real criterion of obesity had relatively lower sensitivity and higher specificity, i.e., BMI had less capability in recognition of obese individuals correctly and higher capability in recognition of individuals with normal weight as compared with FMI [22].

In the study by Haeri-Behbahani, the 90th percentile values of FMI for 6-11 years children were reported as 5,2, 5,9, and 5,6 (kg/m^2) for boys, girls, and total children, respectively. When FMI as the real criterion of obesity was applied, BMI sensitivity and specificity at equal to or more than the 95th percentile of the CDC 2000 standard for determining obesity were reported to be 43.3% and 99.4%, respectively, and the difference observed at the obesity level based on these two criteria was significant. Based on the results of that study, BMI had lower performance in obesity diagnosis in children and FMI was a better criterion than BMI for obesity evaluation in children [22]. In the study of Eto *et al.*, the validity of BMI and FMI was evaluated by considering body fat mass of more than 20% and 25% in boys and girls, respectively, as the real criterion of obesity in children and also determining the 90th percentile of the data obtained from calculating BMI and FMI for defining obesity. Thus, sensitivities of BMI and FMI were calculated as 37,5% and 68,8% in boys and 30,4% and 42,9% in girls, respectively. In

their research, FMI showed higher sensitivity than BMI but both indicators demonstrated lower capability than body fat percent for diagnosing obese children. In addition, specificities of BMI and FMI were calculated as 95,5% and 99,5% in girls and 96,4% and 100% in boys, respectively, both of which showed high specificity [22, 28]. Due to observing a correlation between BMI and FMI on the one hand and body fat percent on the other, this study suggested both BMI and FMI as indicators of fat mass.

The study by Demarath et al. which was conducted on 494 girls and boys within the age range of 8-to18-year-old showed that FMI significantly increased only at high percentiles of BMI. Although means for BMI were similar in girls and boys, FMI was significantly different in the two genders. In this study, with the equal increase in BMI percentile, body fat increase with age in heavier girls was higher than lighter ones. This research concluded that changes in BMI percentiles in children might not properly show changes in body fat mass in the course of time, especially in boys with low BMI [24].

Based on the results of his study on 5-to18-year-old individuals, Freedman stated that BMI accuracy as the estimation of body fat mass greatly depended on obesity intensity so that it had high correlation with FMI in children with the BMI more than the 85th percentile and high correlation with fat free mass in children with the BMI less than the 50th percentile. As a result, BMI difference in thin and normal children could arise more from body fat free mass [25]. In Colombo, study agreement between measured BMI and determined FMI based on DXA method was evaluated. The result showed 75% of the underweight subjects had normal FMI. Thirty percent of the subjects who were normal weight based on BMI had high body fat. In overweight subjects, 6.7% had normal FMI and 40% had very high fat mass. This research concluded that there was good agreement between BMI and FMI and moderate agreement between BMI and the body fat percentage and metabolic syndrome risk [22].

The use of FMI, FFMI, and LBMI in children is limited due to a lack of robust reference data [30]. There is an ongoing need to perfect methods that provide information beyond mass and structure (static measures) to kinetic measures that yield information on metabolic and biological functions. On the basis of the wide range of measurable properties, analytical methods and known body composition models, clinicians and scientists can quantify a number of body components and with longitudinal assessment, can track changes in health and disease with implications for understanding efficacy of nutritional and clinical interventions, diagnosis, prevention, and treatment in clinical settings. With the greater need to understand precursors of health risk beginning in childhood, a gap exists in appropriate in-vivo measurement methods beginning at birth [39].

Conclusions

The fact that body mass index represents only a crude proxy for body fat and may produce a significant level of misclassification, but in the absence of alternative measures, the advantages of body mass index have outweighed its disadvantages. However, bio-impedance offers the opportunity to move beyond body mass index. Its advantages are that it is relatively inexpensive, portable, simple and rapid to use. Its disadvantages are that it is less accurate than more sophisticated methods. Further studies to elucidate the relationship among BMI, body fatness, fat distribution, and various diseases and health risks in children and adolescents should be followed.

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Recommended options in preventing the postpartum hemorrhage

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Abstract

Background: Postpartum hemorrhage (PPH) is a major public health problem due to complications that have a direct impact on the most important reproductive health indicators: maternal morbidity and mortality. It is a leading reason for peripartum hysterectomy, admission of pregnant women to intensive care units, and massive blood transfusion. In the most severe cases, hemorrhagic shock may lead to anterior pituitary ischemia, occult myocardial ischemia, dilutional coagulopathy. Post-partum anemia increases the risk of post-partum depression. To decrease the incidence of postpartum hemorrhage the conditions that are known to be associated with PPH and these women should be identified. Careful anamnesis and obstetrical examination should be done to screen for risk factors in pregnancy and labor. To assure minimal blood loss during delivery several measures are proposed in international protocols. The knowledge of prophylaxis measures is useful to reduce maternal mortality and morbidity from PPH.

Conclusions: The knowledge of prophylaxis measures is useful to reduce maternal mortality and morbidity from PPH. Actually, for prophylaxis of postpartum hemorrhage, evidence based medicine recommend correction of anaemia, use of the partograph in labor and the close supervision first two hours after the delivery.

Key words: postpartum hemorrhage, risk factors, active management, labor.

Introduction

Postpartum hemorrhage (PPH) is the leading cause of maternal mortality in all over the world and a major cause of severe acute maternal morbidity even in well resourced settings [1, 2]. It is a leading reason for peripartum hysterectomy, admission of pregnant women to intensive care units, and massive blood transfusion. In the most severe cases, hemorrhagic shock may lead to anterior pituitary ischemia, occult myocardial ischemia, dilutional coagulopathy [3, 5]. Post-partum anemia increases the risk of post-partum depression [4]. The majority of these maternal complications can be avoided through the active management of the third stage of labor, correct diagnosis of postpartum hemorrhage and adequate treatment [5-8].

The uteroplacental circulation starts with the maternal blood flow into the intervillous space through decidual spiral arteries (fig. 1).

During placental separation, these vessels are tied off the implantation site that induces bleeding from placental site. Reduction in uterine volume after the delivery of the neonate is the result of:

- Strong third-stage contractions
- Placental delivery
- Myometrial contraction
- Compression of spiral arteries
- Clotting and obliteration of the lumen of spiral arteries in the superficial myometrium at the placental implantation site.

This is why fatal postpartum hemorrhage can result from uterine atony. Adhered placental pieces or large blood clots that prevent effective myometrial contraction will also stimulate bleeding.

Definition. On average, women will lose about 500 ml of blood in a vaginal delivery, 1000 ml in cesarien section, and 1500 ml in a cesarean hysterectomy [9, 10, 11]. In 2012 WHO defined Primary Postpartum Haemorrhage as a blood loss of 500 ml or more within 24 hours after birth. In other guidelines and protocols primary PPH is commonly defined as excessive blood loss of 500 ml or more within 24 hours after the delivery of the baby (after the second stage of labor) in a vaginal delivery, and 1000 ml in a cesarean section [9, 10, 14, 18].

Risk factors. Unfortunately, PPH is a complication that for many women cannot be predicted. There are, however, some conditions that are known to be associated with PPH and these women should be identified. Risk factors for PPH may present antenatally or intrapartum; carefull anamnesis and obstetrical examination should be done to screen for risk factors in pregnancy and labor [11, 12]. The following risk factors for PPH are: anemia diagnosed in the beginig of pregnancy, past history of severe PPH, anticoagulant drugs in pregnancy, severe pre-eclampsia, HELLP syndrome, ute-

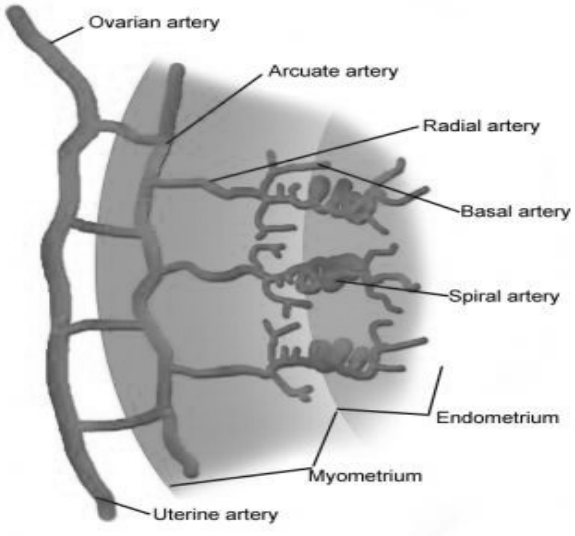

Fig. 1. Schematic presentation of the endometrial vascular system. The primate endometrium is composed of the stratum basalis and stratum functionalis. Uterine arteries branch within the myometrium to yield the arcuate and radial arteries.

rine fibromas, multiple pregnancy, fetal macrosomia, obesity BMI>35, general anaesthesia, failure to progress in the second stage, prolonged third stage of labor, retained placenta, placenta accreta, episiotomy, perineal laceration [13-16]. Clinicians must be aware of risk factors for PPH and should take these into account when counselling women about place and modality of the delivery. Women with significant risk factors for PPH should deliver in a unit with rapid access to blood and blood products. Women with known risk factors for PPH should only be delivered in a hospital with a blood bank on site [17, 18].

Prophylaxis. In order to minimize the risk of PPH we have to reduce blood loss during delivery.

To assure minimal blood loss during delivery several measures are proposed in international protocols:

1. Correction of anaemia.
- Screening for anaemia at the first antenatal visit and appropriate investigations should be offered to all pregnant women [19, 20]
- The aim of antenatal care is to ensure a haemoglobin level at 110 gms/dl
- In order to prevent anemia iron and folate supplementation should be offered to all pregnant women
- The most common cause of anaemia in pregnancy is iron deficiency [21, 22]
- Iron should be replaced orally or by iron infusion. Parenteral iron will be given if oral iron is not tolerated or not absorbed or the term is approaching and there is no time for oral supplementation to be effective [23-24]
- If hemoglobin level is less than 8gms/dl further investigation should be done to diagnose the cause of anemia [25]. Hemoglobin level should be checked in all women at the onset of labor. If less than 10gms/dl, they should be referred to continue labor in the referral hospital where blood is available on site [26, 27].

2. Identification of patients who refuse blood transfusion. Some women refuse the use of blood products for religious pregnant women may refuse blood transfusion because of their religious believes or infection reason. Pregnant women who refuse blood transfusion are at increased risk of severe morbidity, maternal death and perinatal complicatins [28, 29, 30]. In these patients all the steps should be done to minimize the risk of PPH. Antenatal care and delivery in a level 2 or 3 hospital under specialist supervision and specialist consultation at haematology department [31]:
- Optimization of iron stores with haematics
- Delivery in the facility with availability of Haemopure (a blood analogue)
- Delivery in the facility with availability of auto transfusion (cell salvage technique)

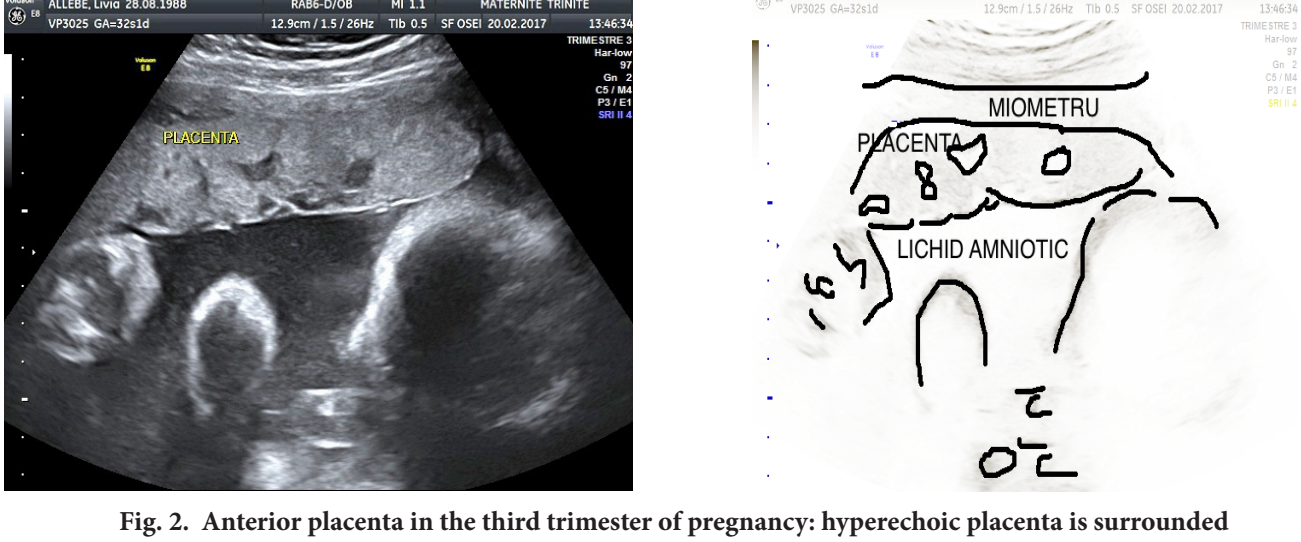

Fig. 2. Anterior placenta in the third trimester of pregnancy: hyperechoic placenta is surrounded by the hypoechoic myometrium, some calsifications and vascular lacks are seen.

- Early use of uterotonic agents, oxytocin 5 iu by slow intravenous injection for prophylaxis in the context of caesarean delivery and for women delivering vaginally, oxytocin 10 iu by intramuscular injection is the regimen of choice for prophylaxis in the third stage of labour. Intramuscular oxytocin should be administered with the birth of the anterior shoulder, or immediately after the birth of the baby and before the cord is clamped and cut.
- Early application of surgical procedures to stop the hemorrhage [32-36].

3. Determination of placental localization via ultrasound (fig. 2).

- Recognition, preparation and management of women with placenta praevia decrease the risk of catastrophic haemorrhage.
- Patients with suspected morbidly adherent placentation should be detected before delivery in order to organize the delivery in the specialized units [37, 38].

4. Use of oxytocin and prostoglandins. Induction of labor should be done with precautions and with strict indications because using excessive dose of prostaglandins and oxytocin can lead to uterine rupture. Induction with prostaglandins in the presence of uterine activity can provoke uterine. Inappropriate use of oxytocin if feto-pelvic disproportion or malpresentation are suspected can lead to uterine rupture [39, 40, 41].

5. No to the "Active management of the third stage of labour". The administration of a prophylactic uterotonic after the delivery of a baby and the controlled cord traction are recommended.The agent of choice for prophylaxis in the third stage of labour is oxytocin10 iu by intramuscular injection [42, 43, 44]. In comparison with the active management, the expectant management involves waiting for signs of placenta separation and spontaneous delivery. Compared with expectant management, the active management of the third stage of labour is associated with a substantial reduction of PPH [45,46]. Active management of the third stage includes routine early clamping of the umbilical cord. A systematic review showed that delaying clamping for at least 2 minutes is beneficial to the newborn and that the benefits extend into infancy [47, 48]. That is why active management of the third stage of labour can no longer be recommended [49].

6. Routine episiotomy. Episiotomy means incision of the perineum in order to enlarge the vaginal orifice during the second stage of labor. It can laterally be done midline or medio-lateral. There are routine episiotomy that is done for every birth and restrictive episiotomy- only under indications. Restrictive episiotomy results in less severe perineal trauma. Restrictive episiotomy results in less posterior perineal trauma, less suturing and fewer PPH rates [50, 51, 52].

7. Use of partogram in labour. The partograph is a graphic record of the progress of labour and relevant details of the mother and fetus. Use of partogram helps to assure appropriate care and monitoring of women in labour because it is an early warning system to detect labour that was not progressing normally [53, 54]. The partograph has been shown to be effective in:

- Prevention of prolonged labor with the associated PPH due to the uterine atony,
- Prevention of prolonged labor with the associated PPH due to the chorioamniotis,
- Prevention of prolonged obstructed labor- the uterine rupture and associated catastrophic haemorrhage,
- Prevention of scar dehiscence and uterine rupture in case of women having labour after one caesarean section [54, 55, 56].

8. Close monitoring during first 2 hours after birth. Following delivery all women will be observed in the delivery room for 2 hours. The observation include her general physical condition, as shown by her colour, respiration and her own report of how she feels, the vaginal blood loss, blood pressure, heart rate. Early detection of PPH before blood loss has become severe and will result in earlier initiation of resuscitation and definitive treatment to arrest haemorrhage, thus reducing the morbidity from the PPH [58, 59, 60].

Diagnosis. The diagnosis of PPH is established by observing the amount of bleeding and the patient's clinical status. Physiological increase in circulating blood volume during pregnancy means that the signs of hypovolemic shock become less sensitive [61, 62].

Tachycardia, tachypnoea and a slight recordable fall in systolic blood pressure occur with blood loss of 1000–1500 ml. A systolic blood pressure below 80 mmHg, associated with worsening tachycardia, tachypnoea and altered mental state, usually indicates a PPH in excess of 1500 ml [63, 64, 65].

Blood loss estimation. We have taken into consideration that estimation of blood loss is notoriously inaccurate, especially with excessive bleeding. Also, physiological changes of pregnancy may mask the severity of blood loss. Clinical signs and symptoms should be included in the assessment of PPH. Estimation of blood loss should begin immediately after the infant's birth and prior to delivery of the placenta. Urinary catheter should be placed as soon as possible. All blood-soaked materials and clots should be taken into consideration to determine cumulative volume. 1 gram weight = 1milliliter blood loss volume [67, 69, 70].

Methods of measuring blood loss were divided into five categories:

- ✓ Visual estimation,
- ✓ Direct measurement,
- ✓ Gravimetric,
- ✓ Photometry,
- ✓ Miscellaneous methods.

For this purpose a basin in front of the external genitalia, a douche pan, underbuttocks drapes with a graduated pouch for measurement, weigh sponges and suction contents placed on a scale can be used.

Clinical signs of hemorrhage are: cool extremities, decreased urine output, dizziness, marked pallor, hypotension with sitting, anxious state, oliguria / anuria, agitation, confusion, loss of consciousness, unstable blood pressure [71,72,73].

Causes of PPH: The modern approach to the postpartum haemorrhage implies the 4T approach (tab. 1) that means implications of 4 factors in the realisation of haemostasis [74, 75, 76].

These 4 T of obstetric hemorrrhage are:

1. Tone (Uterine atony).
2. Trauma (Genital trauma including damage to vulva, vagina, cervix and uterus).
3. Tissue (Retained and invasive placenta).
4. Thrombin (Coagulopathy).

Table 1

"4 T" causes of post-partum hemorrhage

Tissue. Placental causes: Retained placenta Retained clots Placenta previa Placenta accreta/increta/percreta Hydatiform mole	**Tone.** Uterine atony. Hyperstimulation High parity Obstructed labor Early preterm labor Uterine overdistention Large fetus- estimated fetal weight > 4kg Multiple fetuses Hydramnios Labor induction Anesthesia or analgesia Halogenated agents Conduction analgesia with hypotension Labor abnormalities Rapid labor Prolonged labor Augmented labor Previous uterine atony Chorioamnionitis Sepsis syndrome
Trauma. Injuries to the birth canal Episiotomy and lacerations Forceps or vacuum delivery Cesarean delivery or hysterectomy Uterine rupture Previously scarred uterus Intrauterine manipulation Midforceps rotation Breech extraction Shoulder dystocia	**Thrombin.** Coagulopathy Congenital coagulopathies Placental abruption Sepsis syndrome Severe preeclampsia Syndrome Acute fatty liver Anticoagulant treatment Amnionic-fluid embolism Prolonged retention of dead fetus Massive transfusions

If the placenta is out, the most common cause is uterine atony, diagnosed by palpation of a poorly contracted uterus. This is first done by knowing if the placenta has been delivered or not. If retained then the management follows along the retained placenta route in the algorithm. Perineal tears will be diagnosed on routine inspection after vaginal delivery. If there is no response to oxytocin infusion additional oxytocic agents should be given as indicated in the algorithm. First line is represented by ergometrine and misoprostol, but intramyometrial PGF2alpha requires a special indications to administer. However at this stage, in the presence of persisting bleeding, it is very important to exclude another cause such as deep vaginal lacerations, cervical tears and/or retained fragments of placenta or membranes.

Conclusions

The knowledge of prophylaxis measures is useful to reduce maternal mortality and morbidity from PPH. Actually, for prophylaxis of postpartum hemorrhage, evidence based medicine recommend correction of anaemia, use of of the partograph in labor and the close supervision first two hours after the delivery.

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About causes of early-stage asymptomatic prostate cancer

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Abstract

Background: The neurotransmitters (epinephrine and norepinephrine) of the sympathetic nervous system that perform numerous cellular and tissue functions contribute to tumor growth during the early stages of development. At the same time, these bioactive substances act as mediators of the descending antinociceptive system that cause inhibition of pain at the suprasegmental and segmental levels of the neurotransmission. Later studies point to the involvement of afferent sensory neurons in tumor process. The functionality of these structures can be changed due to the structural features caused by genetic disorders of myelin. In addition to that, tumor augmentation of sensory neurons endings leads to the involvement of myeloid-derived suppressor cells in the affected area and the creation of an immunosuppressive microenvironment. At the same time, in the secondary inflammatory process, various enzymes that change the cellular matrix and cause invasion and metastasis are released. In addition to sensitizing cytokines, immunocompetent cells – macrophages, neutrophils, lymphocytes – can also produce opioid peptides that target the desensitization of peripheral nociceptors. Opioid peptides inhibit the excitability of sensory nerves without central unwanted side effects such as depression of breathing, clouding of consciousness, or addiction. This peripheral antinociceptive system with ICC may allow the neoplasm to remain asymptomatic for a while. The changes in afferent impulses at the central level in oncopathology can also be associated with those in the functionality of Toll-like receptors.

Conclusions: Taking into account the aforementioned literature data about oncogenesis, it may be assumed the presence of a new complex pathogenetic pattern that ensures the asymptomatic evolution of prostate cancer. A better coverage of this data may facilitate further search for early markers of the disease.

Key words: prostate, cancer, asymptomatic.

Introduction

Prostate cancer (PCa) is the most common cancer type among the male population of Europe and it is on the third place in the structure of oncological diseases. In 2012, about 417,000 new cases of cancer were diagnosed, out of which PCa represents 12% of the total number of cases [1]. Castration-resistant prostate cancer (CRPC) is second among the main causes of death from malignant diseases in representatives of the stronger sex [2].

According to pathoanatomical studies, it was found that prostate cancer has a long period of asymptomatic growth. It sometimes takes several decades before the appearance of a clinically pronounced form. In the opinion of Sakr W. A. et al. [3], small foci of histological cancer were detected in 27% and 34% of 40 and 50-year-old men, respectively. According to Breslow N., et al. 1977, Barry M. J. 2001 [4-5] , prostate cancer appears in 60% - 80% of men older than 70 years and only in 0.1% of individuals aged under 50 years [6].

Despite such a high prevalence, the latent form of prostate cancer becomes clinically significant only in 10% of cases [7]. Most scientists share the view that late detection of prostate cancer is associated with a prolonged asymptomatic evolution of the disease. The study of the dynamics of cancerous tumours growth has shown that ¾ of time for their development falls on the preclinical period [8]. In the opinion of B. Ya. Alekseev et al., metastases are found in 60-80% of patients with primary prostate cancer [9].

Until the 1970s (pre-screening era), in Western countries, the late stage (metastatic prostate cancer) in primary diagnosis was observed in more than 50% of cases. In the present period, 10 to 20% of patients also have distant metastases at the time of diagnosis [10].

Authors who have studied the features of PCa with fatal outcome provide similar data. According to them, PCa metastases were identified in 56% of patients who died of prostate cancer [11]. The differential diagnosis of PCa is carried out with the other prostate diseases, previous ineffective drug therapy of LUTS [12].

Analyzing the scientific data on the tumour microenvironment and its interaction with various macroorganism systems, some features can be outlined, which may show that a tumour can remain asymptomatic for a long time.

Interaction of nerves and tumors

Experimental models have relatively recently shown the involvement of nerve fibres in the tumour tissue in prostate cancer [13]. Cancer cells can invade nerves surrounding the tumour by expression and secretion of nerve growth factor (NGF) [14]. The ability of NGF to regulate expression and production of neurotransmitters, as well as modulation of synaptic activity [15] were also detected. In their turn, antihyperalgesic and analgesic effects may be accompanied by changes in synaptic transmission [16-17].

The original work on the interaction of malignant neoplasm and those involved in the pathological process of afferent (sensory) neurons was performed by the authors [18].

In the course of the study, they revealed that there is tumor augmentation of endings of sensory neurons leading to hyperproduction of chemokines by nerve cells of the dorsal root ganglia (DRG). According to scientists, the formed tumor-neuronal-immune axis promotes the involvement of myeloid-derived suppressor cells (MDSC) in the affected area and the creation of an immunosuppressive microenvironment.

According to Magnon C. et al. [13], the nerve fibres of the sympathetic nervous system "contribute" to prostate cancer in the early stages of development by producing the neurotransmitter "norepinephrine" (NE). Observations of scientists [19-20] point to the dominant role of norepinephrine locally secreted by nerve fibres in controlling beta-adrenergic effects on tumour development. On the other hand, NE is also a mediator of the descending antinociceptive system by activation of adrenergic receptors that causes inhibition of pain at the suprasegmental and segmental levels of the neurotransmission [21].

Abnormal architectonics of tumour tissue is reflected in the components of neurovascular structures. According to some researchers [13, 14, 22], differences between the growth of nerve cells in normal tissue and tumours can be detected. In a tumour, the axon lengthens, whereas in normal tissue the cell body of the neuron thickens. Moreover, some authors report the correlation between the density of nerve fibres within prostate carcinoma and the degree of its aggressive behavior [23, 14].

Studies on prediction of upgrading and disease upstaging in low-risk prostate cancer identify a panel of three genes which expression significantly affects the aggressive behavior of the disease [24]. One of these genes, PMP22, encodes glycoprotein contained in ~ 5% of the total myelin protein in the nervous system [25, 26], while genetic defects related to PMP22 are also associated with peripheral neuropathy [27]. The above features may affect the transaxonal transport. According to the author [28], the violation of the latter mechanism can lead to a decrease of mediator content in the presynaptic structures and create an analgesic effect. This principle – decrease of mediator content in presynaptic structures – underlies the action of anaesthetics.

Features of the metabolism of blastemal cells and nociception

The atypism of tumour tissue, as noted above, affects the morphology of the constituent nerve structures, a fact that may also change their functionality. Further, we will present some features of blastoma cells that may influence the intensity of pain impulses from the oncologic focus.

An increase in the proliferative activity of the cell in response to mitogenic stimulation is accompanied by a large-scale dynamic increase in the concentration of cytosolic calcium [29]. For example, the authors [30], examining the level of cytosolic calcium (Ca2+) oscillations in oesophageal squamous cell carcinoma (ESCC), have noted significantly higher indices of this parameter in blastemal cells in comparison to the control ones – 76% versus 26%, respectively.

The change in intracellular calcium concentration involves the inducing of a whole cascade of intracellular events, including the activation of transcriptional and apoptotic mechanisms [31, 32, 33].

The possibility to avoid the inclusion of the mechanisms of apoptosis provides one of the fundamental conditions of oncogenesis – the possibility of uncontrolled division. Tumour cells can avoid apoptosis by decreasing the cytosolic calcium concentration [34, 35]. The latter phenomenon can be achieved by changing the functionality of the membrane Ca2 + ion channels [36]. Some features of these structures have been revealed in the course of PCa [37, 38].

TRPM8 is one of the Ca2 + ion channel groups, and has been first identified in PCa cells, but it was found later that TRPM8 channels are also expressed in nociceptive neurons [39].

Some interesting features are noted by increasing the range of studies in terms of analyzing the consequences of disruption of the calcium channels. According to the feedback mechanism in the peripheral painful systems of Ca2 +, the ion channels are activated low and inactivated by a high concentration of cytosolic calcium [40]. According to the author [41], the suppression of calcium currents by 20-90% from the initial values (depending on the type) is one of the components of the local anaesthetic effect of tetracaine. Scientists [42, 43, 44, 45] provide similar data – blocking ion channels can ensure terminating the action potential (AP), with the establishment of local anesthesia. In this context, the authors' conclusions [46] that the decrease in the transmembrane Ca + transport facilitates the establishment of analgesia also enhances the effect of opioids.

Reduction of the axon excitability and nerve endings in some neurons has also been observed in the hyperfunction of calcium-activated chloride channels (CaCCs). These structures are expressed in excitable and epithelial cells, ensuring stabilization of the resting membrane potential and cell volume regulation. The number of functioning nerve endings is regulated by modulating conductivity of chlorine. For example, the activation of CACCs reduces the normal excitability and facilitates the establishment of a block for carrying out the action potential in the branch node [47, 48].

Investigating the impact of neuroendocrine differentiation in PCa cells on the characteristics of the volume-regulated chloride channels, Lazarenko R. N. [49] has revealed a 2-fold excess of this parameter in comparison to the control level.

Some of the above theoretical considerations have been practically confirmed in various experiments on animals. It was found that modulation of both central and peripheral ion channels could significantly change the pain sensitivity threshold. Researchers [51, 52, 53] have established signs of a decrease in pain sensitivity in experimental rodents with impaired permeability of calcium channels. An interesting fact is that the increased Ca2+ influx is considered critical for the transmission of persistent but not short-term pain

impulses [54]. The noted features can be manifested in cancer patients because of local ionic disorders.

An atypical metabolism with a predominance of glycolysis is one of the main logos of carcinogenesis. During rapid replication of tumour cells, it is necessary to have a huge amount of biomaterial for the synthesis of cellular structures, which to some extent can be provided by anaerobic glycolysis [55]. At the same time, this feature of the exchange is ineffective in terms of ATP production [56]. The production of ATP is only 50% of the total level in the mitochondria of malignant cells, whereas in conventional cells this figure reaches 90% [57]. The latter feature may possibly affect the level of this substrate in blastemal cells, followed by a violation of purinergic signal transmission between neurons in the tumour environment and its microenvironment [58]. According to Fields R.D., et al., the release of neurotransmitters in the peripheral nervous system (PNS) occurs with the assistance of ATP and adenosine [59], and also processing of sensory information [60] is provided by means of these mediators by purinergic receptors.

Perversion of metabolic processes characteristic to cancer cell degeneration is accompanied by a decreased activity or the absence of certain specialized enzymes inherent in normal tissues (arhipase, catalase, cytochrome oxidase, cytochrome c, esterase, etc.) [61]. For example, researchers [62, 63] indicate a decreased expression of Na +, K + -ATPase in some carcinomas. Na+ /K+ -ATPase is a necessary enzyme to maintain sufficient activity of the Na+/K+) pump – one of the key processes of vital activity involved in the regulation of cellular metabolism, water-salt metabolism, as well as to generate excitation. The activity of Na / K-ATPase is dependent on the ATP content in the cell [64]. A decrease in Na+, K+-ATPase activity leads to slower nerve impulses and may be accompanied by a loss of pain sensitivity [65].

Another mechanism that contributes to the reduction of nociception in the early stages of cancer is probably related to a change in the functionality of the cyclase systems. In particular, some solid tumours show a deficiency of adenylate cyclase in the intermembrane space [61]. The authors [66, 67] have noted the direct inhibitory effect of calcium ions on isoforms 5, 6 of adenylate cyclase. In the light of the above data on the increase of cytosolic calcium level in blast cells, remarks of the authors [68] who report a marked decrease in acute and chronic pain intensity via blocking adenylate cyclase activity in animal models are very interesting.

Immune system and sensory influences

One of the most significant milestones of modern immunology is the formation of a scientifically based concept of innate and adaptive immunity. From an evolutionary point of view, innate immunity is an earlier protective mechanism inherent in virtually all multicellular organisms. Being hereditary, this structure provides protection of the individual from various microorganisms and endogenous derivatives of tissue disintegration, activating for several minutes or hours. All components of innate immunity are invariably inherited and are not genetically modified throughout life. Functioning of this protective system is provided by numerous cellular elements (eosinophils, mast cells, macrophages, neutrophils, basophils, NK cells), microglial cells – resident macrophages of the central nervous system (CNS) and humoral factors (lysozyme, cytokines, complement, acute-phase proteins (APPs), cationic antimicrobial peptides, etc.) [69]. C. Janeway formulated the principle of innate immunity at the end of the 20th century by introducing the concept of pathogen-associated molecular patterns (PAMPs), which are encoded in the genomes of bacteria and absent in the genome of macroorganisms. The most studied PAMPs are DNA and RNA viruses and bacteria, flagellin, bacterial wall lipopolysaccharides (LPS), glycolipids, lipoteichoic acid (LTA), lipoproteins, zymosan fungi [70, 71]. It has also been found that many macroorganism-derived compounds formed during cytolysis can act as PAMPs (fibrinogen, heat shock proteins, fibronectin, etc.) – damage-associated molecular patterns (DAMPs) [72, 73, 74]. Identification of antigens by the cells of the innate immune system is carried out by receptor formations that distinguish the pattern of pathogens (pattern recognition receptors – PRRs). Pattern-recognition receptors (PRRs) are divided into three classes according to their function: signaling, endocytic and secreted. Signaling PRRs contribute to the transmission of the signal into the cell nucleus to activate the genes of adaptive immunity. Endocytic PRRs mediate the damage of the pathological agent in the lysosomes of macroorganism cells. Secreted PRRs act as opsonins, "marking" antigenic structures and contributing to the process of phagocytosis [75]. One of the most significant elements of the class of PRRs are Toll-like receptors (TLRs) [76]. These structures were first discovered in 1997 in mammals [77]. Receptors of this class are widely represented in various cells of organs and body systems (monocytes, leukocytes, fibroblasts, endothelium, epithelium, cardiomyocytes, B cells [78], mast cells [79], natural killer cells (NK cells) [80]. TLRs are common in various cell populations of the central nervous system (CNS): dendritic cells (DCs) [81], neurons [82], astrocytes [83] and oligodendrocytes [84], and glia [85]. This feature provides a significant link between the innate immune system and the CNS. The abundance of TLRs in pain responsive regions makes them a critical potential component of pain signaling.

Glia is a collection of accessory cells of the neural tissue, accounting for more than 70% of all cells found within the brain and spinal cord [86]. Glial cells have been recognized as key mediators of the innate immune responses in the CNS and play a major role in the clearance of cellular detritus and immune surveillance [86, 87]. It should be noted that complementary glial cells are important modulators of pain. According to scientists Piccinini AM. et al. [89]; Scholz Z., et al. [90], damage-associated molecular patterns (DAMPs) can activate glial cells through TLR receptors, which have a well-established role in pathological pain. On the other hand, it has been proven that some tricyclic compounds that are commonly used for clinical neuropathic

pain treatment possess significant TLR4 inhibitory activity and can reduce sensitivity (Hutchinson MR, et al.,) [91]. In preclinical experiments, the study of pain mechanisms revealed interesting features of expression of TLRs [92]. After the induction of peripheral inflammation (plantar administration of Complete Freund's Adjuvant (CFA) in laboratory rats), the transcriptional level of mRNA TLR4 significantly increased within a short period of time (4 hours) in various regions of the central nervous system. This indicator has remained high for 14 days, and persisted even when the emerging signs of experimental allodynia disappeared. In this context, I would like to mention the remarks of researchers [93], who noted the activation of microglial cells as a result of peripheral nerve damage (peripheral neuropathy). The authors noted an interesting feature of glia – once activated microglial cells can remain in a "sensitized" state. Similar changes in the properties of glial cells were noted not only as a result of peripheral nerve damage, but also as a result of stress factors [94]. According to Ferraz CC. et al. [95], Diogenes A. et al. [96], the functionality of TLR4 depends on the amount of intracellular calcium and the level of sensitization of TRPV1.Given the above information about the ability of tumor cells to suppress the functionality of membrane calcium channels [37], we can assume a local (tumor) inhibitory effect on TLR systems. Perhaps this phenomenon ultimately operates systemically, inducing a special "hyposensitized" state of glia. Probably the indirect confirmation of this assumption is the observations of scientists Tashiro M, et al. [97], who studied the brain of 19 patients with various types of cancer (except for brain cancer) using positron emission tomography. The results of the study were compared with images of 17 patients with benign diseases. The authors noted a decrease in regional cerebral glucose metabolism in separate areas of the CNS – limbic system, thalamus, hippocampus, basal ganglia, etc. According to them, the psychological deficit in cancer patients is associated with abnormalities of regional brain metabolism in the limbic system. Given the specifics of our research, we consider it worthwhile first of all to note the reduction of regional cerebral glucose metabolism in the thalamus. This structure not only retransmits all sensory and motor information from the sense organs, but also performs primary processing and thus filters the incoming sensory information before transferring it to the cortex of the large hemispheres [98]. The given changes in such an important area of the central nervous system as the thalamus most likely have a negative impact on its functionality and lead to sensory disorders.

Recent studies indicate the probability of synthesis and secretion of identical regulatory peptides (substance P (SP), VIP, enkephalins, cholecystokinin, somatostatin, beta-endorphin, lipotropins, angiotensin, calcitonin) by cells of various organs and tissues. The affinity of these compounds to the receptors has been discovered, and it looks common in many body systems. Considering the latter, substitution of the concept of nervous, immune, endocrine and humoral modulation by the term "regulatory continuum" has been proposed [99].

According to the postulates of integrative medicine, the conjugated interaction of nociception and immunity now occurs both at all levels of the nervous system, and in all organs and components of the immune system. In the last process, almost all known hormones, neurotransmitters and cytokines are involved. Thus, the receptors of neurotransmitters involved in the occurrence and conduct of pain impulses also affect the functionality of immune cells. At the same time, a number of hormones, cytokines and other bioactive compounds secreted by lymphoid cells, changes the excitability of nerve fibres [100].

Early studies on the role of the immune system in the development of cancer indicate a circular infiltration of immune cells by tumours [101, 102, 103]. The authors have convincingly demonstrated tumour stimulation by immune system cells and neoplastic progression [104, 105]. Stimulation of these structures occurs as a means of adrenergic influences of macroorganism and cytokines produced by a cancerous tumour [106, 107]. The suppressor effect in prostate cancer on the population of T-killers [108] was also proven. In the inflammatory process, the release of proangiogenic factors and enzymes that change the cellular matrix and promote invasion and metastasis occurs in infiltration of the tumour microenvironment by immunocompetent cells [109].

On the other hand, besides these sensitizing cytokines, immunocompetent cells (ICC) – macrophaghes, neutrophils, lymphocytes – can also produce opioid peptides that provide desensitization of peripheral nociceptors [110, 111, 112, 113].

Inflammation of peripheral tissues leads to increased synthesis and axonal transport of opiate receptors in dorsal root ganglion neurons, which causes an enhanced analgesic efficacy of peripherally active opioids. Once secreted, opioid peptides activate peripheral opiate receptors and produce analgesia by inhibiting the excitability of sensory nerves. These effects occur without central untoward side effects such as depression of breathing, clouding of consciousness, or addiction [114, 115]. This peripheral antinociceptive system with ICC may allow the neoplasm to remain asymptomatic for a while. In this context, we consider interesting the observations of the authors [116] who have noted that Mycobacterium tuberculosis activates formyl peptide receptor (FPR) on neutrophils, resulting in tonic secretion of opioid peptides from neutrophils and in a decreased inflammatory pain.

Earlier it was suggested that malignant tumour formation is analogous to a certain "killer organ" [117, 118]. Successful progression of this process is provided with clearly outlined strategies. Some of the most well-known strategies are changes in the microenvironment by isolating specific metabolites and tumour secretion of growth factors, growth of malignant blastemal cells during the deterioration of medium conditions, immune-suppression by developing an

antigenic simplification, divergence and antigenic reversion [119]. As is known, a long asymptomatic flowing provides oncogenesis with a "special" effect and perhaps outlines an additional strategy.

Conclusions

Taking into account the aforementioned literature data about oncogenesis, it may be assumed the presence of a new complex pathogenetic pattern that ensures the asymptomatic evolution of PCa. A better coverage of this data may facilitate further search for early markers of the disease.

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Priority in classification of cervical fasciae

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Abstract

Background: The neck is divided into two compartments: anterior compartment, which includes the organs with their fascial cellular structures and the posterior compartment, which is constituted of muscles with their fascial sheaths.
Study of cervical fasciae represents major difficulties, because the authors did not synchronize over the time a common opinion about the fascia and terminology classification. In the manuals of anatomy in English, French and Russian the same formations are specified differently.
Conclusions: The authors of contemporary textbooks and scientific articles describe equally the real anatomy of cervical fasciae in the anterior visceral compartment of the neck, where they are located, what structures envelop, how they delimitate the narrow clefts and large spaces between them, but in the different manner and using different terms for the same fascial leaves. Maybe there is no need to give preference to a concrete classification, of the 3 fasciae as in the official Anatomical Nomenclature or of the 5 fasciae as is in the textbook of V. N. Shevkunenko. It is enough to know the synonyms of the fascial leaves and consider their clinical significance in the spreading of infection, for opening the cervical phlegmons and performing the surgical approaches.

Key words: neck, cervical fascia classification.

Introduction

The neck is divided into two compartments: anterior compartment, which includes the organs with their fascial cellular structures and the posterior compartment, which is constituted of muscles with their fascial sheaths.

Study of cervical fasciae represents major difficulties, because the authors did not synchronize over the time a common opinion about the fascia and terminology classification. In the manuals of anatomy in English, French and Russian the same formations are specified differently. Thus, the prevertebral fascia is determined by the French anatomists as being aponeurosis. English anatomists name it – "*alar fascia*" and the Russian literature, which is based on the classification given in the manual of V. N. Shevkunenko (fig. 1) considers that it is correct to name it *fascia prevertebralis,* which participates in the formation of the respective muscle sheaths. Taking into account this fact the neck fascia need to be regarded through the practical approach related to the clarification of the ways of purulence propagation and elaboration of surgical approach methods [1].

It is well known that it is difficult to establish and systemize the number of fasciae on the neck, the fact which is determined by the age, physical development, gender, method of investigation and etc.

Thus, the goal of this work is the elucidation of author's priorities in the study, description and classification of cervical fasciae.

The problem about the cervical fascia has its origin in the 19th century by the initiative of surgeons and not by the initiative of anatomists. Initially namely the surgeons attempted to describe the cervical fascia according to the practical requirements. Once the problem appeared, the following net involvement of anatomists did not bring benefits in the direction of the studied problem and even it made this problem to be more difficult and currently only the behavior of the cervical fascia represents a difficulty for the classification and interpretation of cervical fasciae. Such authors as V. P. Vorobyov (1932) quotes the complete expression, in principal, philosophical or with moral, practical sense in a minimum of intonation of Malgain'a, which became classical: "Cervical aponeurosis – this anatomical chameleon which appears every time in a new form as the result of the nib of each person who has tried to describe it". Even A. D. Pansch (1888) in "Essentials of Human Anatomy" said that the neck fascia is the subject of the anatomy that can be considered as being the most confused [2].

Fig. 1. V. N. Shevkunenko.

Practice is truth criteria

It would seem that the settlement of the problem about the cervical fascia is simple – take the scalpel and prepare the region of neck and investigate. Although, the more researchers "appealed to scalpel", the more differences and contradictions appeared.

The main cause of the divergences and contradictions in the description of the neck fasciae is determined by the lack of common concepts, generally accepted, about the structure of fascia and other connective-fibrous formations. That is why practically each connective-fibrous structure in the working field and the author's will can be named (and it is frequently named) fascia and the passion for the "fasciology" led to the fact that the term fascia was assigned even to typical adventitia – coverings of organs and sometimes even a portion of the organ covering, for example the pharynx (*fascia faringobasilaris*).

Causes of divergences and terminological confusions

1. Incertitude in the concepts of "fascia" from the structural point of view. Criteria like density, gloss, fiber orientation and other qualitative features are not conclusive for the recognition of the connective tissue layer between the muscles and organs as the independent formation – the fascia.

2. Lack of "genetic" relationship, i. e. a single source of origin. So, as to one of fascia (the 3rd of 5), according to the origin is considered as a rudimentary muscle and the other (the 4th of 5) is considered to have its origin from the coelomic epithelium.

3. Features for the relations of fascia sheets – fusion and division. As the consequence one and the same fascia sheet can be considered as an independent fascia and/or a constituent part (sheet, lamella) of another fascia. Thus, from this fact results the number of fasciae – from one to six.

4. There are different "topographical" approaches when the fasciae are described. Thus, if we distinguish according to the depth location the superficial fascia and the deep fascia, then vertically in one of the fasciae we distinguish the suprahyoid and infrahyoid portions.

5. Distinguishing between the neck fasciae the "proper" and "improper" fasciae. Proper fasciae are the fasciae which belong only to the neck and they do not spread out of the neck limits and the improper fasciae are spread in other regions.

6. Usage of different words and at the same time words close in meaning while distinguishing the fasciae – fascia, fascia lamella, fascia sheet, fascia plate, aponeurosis, etc.

7. Small number of studies on fasciae (with using for example the anatomic material) and loss in time of the author priorities. In this way Tonkov described the neck fasciae according to Zernov, Zernov according to Vorobyov, Vorobyov according to foreign authors of 19th century, etc.

8. Exaggeration of the importance of fasciae anatomy for the surgeons and the disappointment of practical doctors in the classifications of the proposed neck fasciae because of the difficulty and complexity of the matter.

Cervical fasciae according to manual of V. N. Shevkunenko

However, which description of neck fasciae should be considered as being original in the proposal of classification according to V. N. Shevkunenko?

For the first time this classification was met in a publication of 1934 [3]. Let's analyze this "first" classification.

1. Chapter "Neck", in which the fasciae are described, was written by Professor V. V. Moskalenko, and not by V. N. Shevkunenko.

2. Initially to fasciae were assigned Latin names, though without linguistic equivalent.

3. In the process of fasciae description the author manifests an unusual precaution for the manual: "Neck fasciae". For the schematic presentation of neck fascial laminae he used his own definitions and some new terms for designation of details, which showed that can be admitted the existence of the five cervical fasciae.

4. In the description of fasciae the author refers to an image which is signed as: "Cervical fasciae according to A. P. Samarin" (fig. 2).

5. In the description of three of five fasciae (the 2nd, the 3rd and the 5th) the author refers to Samarin, Gruber, Richet and other authors. The majority of the authors are quoted according to Samarin.

6. The first neck fascia is considered as the prolongation of the common fascia of the body and it is called *fascia superficialis communis*.

Indicator from the point of view of copyrighted priorities constitutes the description of the third fascia: "the following sheet – *facia colli media Gruber* (or *aponeurosis omoclavicularis Richet*, or the deep lamina of a *fascia colli prorpia* – according to Samarin, or the third sheet according to our current schemes".

In this way in the manual edited by V. N. Shevkunenko the cervical fasciae are primordially exposed "according to Samarin" by Professor V. V. Moskalenko, and the priority of the authors in this manual is that they have just numbered the fasciae and called them sheets – the first sheet, the second sheet, etc.

In the following editions of the manuals of topographic anatomy edited by V. N. Shevkunenko, the references to Samarin, as to other authors, have disappeared, and the chapter "Neck" is not written by Professor V. V. Moskalenko, but by Professor A. Y. Sozon-Yaroshevich [4]. In this way the names of some fasciae were modified. Thus, we will present bellow the author's redaction of the fasciae names in the manuals of 1934 year and (in the brackets) 1951 edition years:

1. First sheet – fascia superficialis communis (fascia colli superficialis).

2. The second sheet – fascia colli superficialis Gruber, superficial lamina of fascia colli propria – according to Samarin (lamina superficialis fascia colli propriae).

3. The third sheet – fascia colli media Gruber, aponeurosis omo-clavicularis Richet, deep lamina of fascia colli propriae – according to Samarin (aponeurosis omo-clavicularis).

4. The fourth sheet – fascia endocervicalis (fascia endocervicalis).

5. The fifth sheet – fascia colli profunda, s. prevertebralis, s. lamina parietalis fasciae endocervicalis – according to Samarin (fascia prevertebralis).

In all four editions, including the fourth postmortem (1943) edition of manuals of human anatomy by N. K. Lysenkov and V. I. Bushkovich [5] the neck fasciae are described "according to A. P. Samarin", there are even indicated images with specification "Cervical fasciae according to Samarin". In the fifth authorized edition of this manual (1958), made by M. G. Prives because of the death of both previous authors (V. I. Bushkovich in 1939 and N. K. Lysenkov in 1941) by the indication of the Ministry of Health of USSR and Medgiz Publishing Company, classification of cervical fasciae "according to A. P. Samarin" has disappeared, and for the first time it appeared the classification "according to V. N. Shevkunenko" [6]. Thus, the names and the description of cervical fasciae in the manual of 1958 edited by M. G. Prives practically repeats word by word as in the manual of 1951 edited by V. N. Shevkunenko.

Fig. 2. Professor A. P. Samarin.

In this way, on the initiative of M. G. Prives after six years of V. N. Shevkunenko's death (1872–1952) it has appeared and it continues to exist in the anatomic literature (especially in the Russian literature) the classification of neck fasciae "according to V. N. Shevkunenko". In this way even V. N. Shevkunenko never has assigned to himself the priority of author in the description and names of cervical fasciae. Even more, in all the editions of the existent manuals edited by V. N. Shevkunenko there are references to Samarin, Gruber, Richet and other authors.

Thus, who is A. P. Samarin, who is in fact the main author, but not the single one, the author of the "5 laminae" classification of cervical fasciae and why his name after 1943 has disappeared from the pages of the manuals and monographs.

A. P. Samarin was professor of anatomy, born in 1874, died not earlier than 1925, the author of the biggest and most original research about the neck description.

In 1922 he was appointed the head of the Department of Topographic Anatomy and Operative Surgery of the University of Medicine of Voronezh, Russia. He came from the University of Medicine of Odessa where in 1912 he defends the PhD Thesis under the title: "Investigation of fasciae and connective tissue spaces of the neck", namely in this thesis he for the first time describes endocervical fascia and demonstrates that it is constituted from the parietal and visceral laminae [7, 11]. The copy of his thesis is kept at the National Library of the Belarus Republic [8].

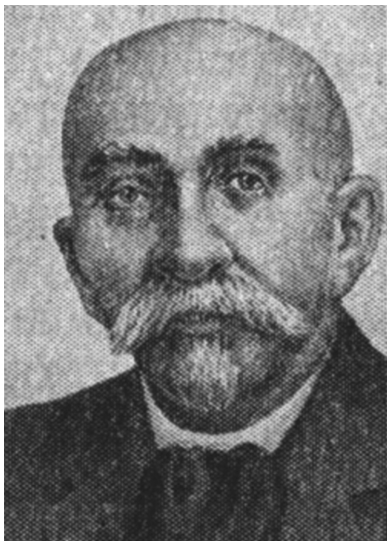

Fig. 3. Professor N. K. Lysenkov.

The title for the PhD was proposed to him by the professor of anatomy N. K. Lysenkov (fig. 3). It is a curious fact that in all editions of the manual of anatomy of the author N. K. Lysenkov in the chapter "The Neck" he refers to his student – A. P. Samarin.

N. K. Lysenkov (1865-1941) was a Russian anatomist and surgeon. In 1893 he finished the faculty of medicine of the University of Moscow; in 1896 he defended the doctor thesis about the cerebral hernia, the theory of formation and its treatment. Since 1902 he was Professor of the Department of Topographic Anatomy and Operative Surgery in the University of Odessa and since 1923 – the head of the department of morphology and physiology [9].

The fate of A. P. Samarin was dramatic and possibly tragic. After the 2nd congress of doctors in Russia, which took place on May 10-14, 1922 in Moscow, through the multitude of the representatives of the dissident intellectuals he was arrested and repressed in the North Siberia. The decisive moment in his arrest was the letter of N. A. Semashko (then he was the commissioner for the health of population), the fact which was certified by the disclosed documents "the doctors repression was coordinated with the commissioner N. A. Semashko" [10]. The fate of A. P. Samarin after the repression is unknown.

Thus, the additional searching for the "correct" names

of neck fasciae and the copyright in their description seem to be inopportune because of the "limitation status", including the incertitude of the main concepts (tissue, fascia, aponeureosis, laminae, plates, etc.). Now the term of "fascia" is unanimously accepted, notwithstanding that it has an indicative character over a concrete structure, but it corresponds sufficiently to the existent idea about fasciae as connective-fibrous coverings of different expression and character – from dense fibrous to thin, lax, cellulous tissue [11].

Now in the anatomy there are kept a lot of vagueness, confusions of terminology, but these historic "mistakes" do not influence significantly the practice. And the "reconciliation" of the parties can be reached by the strict observation of the unique anatomic law – *Nomina Anatomica.*

The international anatomic modern nomenclature (Rome, 1998) – in the composition of a cervical fascia there are three laminae: superficial, pretracheal and prevertebral (it means the 2nd, the 3rd, and the 5th fascia from the list of those five according to the classification of V. N. Shevkunenko. Separately there are distinguished the carotid sheaths and the suspensory ligament of thyroid gland and from the interspascial spaces there is distinguished only the suprasternal space. After the unanimous acceptance of the Parisian International Nomenclature in 1955, the project of the Russian nomenclature elaborated by the commission of Soviet anatomic nomenclature came with the proposal within the International Committee for the Anatomic Nomenclature for the legalization of those five fasciae "according to V. N. Shevkunenko" and adding to this list interfascial spaces because between the cervical fasciae there are narrow clefts and the large spaces. But the International Commission considered these details as being supplementary and has refused the proposal.

Conclusions

The authors of contemporary textbooks and scientific articles describe equally the real anatomy of cervical fasciae in the anterior visceral compartment of the neck, where they are located, what structures envelop, how they delimitate the narrow clefts and large spaces between them, but in the different manner and using different terms for the same fascial leaves. Maybe there is no need to give preference to a concrete classification, of 3 fasciae as in the official Anatomical Nomenclature or of 5 fasciae as is in the textbook of V. N. Shevkunenko. It is enough to know the synonyms of the fascial leaves and consider their clinical significance in the spreading of infection, for opening the cervical phlegmons and performing the surgical approaches.

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Fetal and neonatal complications of diabetic pregnancy

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Abstract

Background: There is currently convincing clinical and experimental evidence that a hyperglycemic intrauterine environment is responsible not only for significant short-term outcomes in the fetus and newborn infant, but it is also an increased risk for long-term outcomes, such as developing diabetes mellitus and other chronic diseases in adulthood. Short-term complications can occur in utero (i. e. diabetic fetopathy, fetal macrosomia, intrauterine growth restriction, congenital malformations, intrauterine fetal death); during labor (shoulder dystocia, birth injuries, intranatal death) and during the neonatal period (respiratory distress syndrome, metabolic, electrolytic and hematological disorders, hypertrophic cardiomyopathy, neonatal mortality). The risk of adverse outcomes is greater in pre-gestational diabetes, but undiagnosed and / or poorly controlled gestational diabetes can lead to similar consequences. Although there is currently a relatively clear view on the pathogenesis of fetal and neonatal complications of maternal diabetes and their interconnections, the deep molecular mechanisms are far from being clearly understood. Furthermore, there has been an unexpected increase in the incidence of gestational diabetes worldwide during the last decades, in association with the obesity pandemic and type 2 diabetes.
Conclusions: Maternal diabetes, especially pre-gestational diabetes has a significant impact on the incidence of fetal and neonatal complications with both short and long-term outcomes.

Key words: pregnancy, diabetes mellitus, gestational diabetes, diabetic fetopathy.

Introduction

All the types of diabetes mellitus(DM) in pregnancy – pre-gestational diabetes mellitus (PGDM) and gestational diabetes mellitus(GDM) are associated with a significantly increased risk of short and long-term maternal, fetal and neonatal adverse outcomes[1, 2, 3]. The risk of developing pregnancy complications is associated with increased maternal blood glucose levels and it is 2 to 5 times higher in women with type 1 diabetes mellitus (T1DM) compared to the general population. Furthermore, there is evidence that type 2 diabetes mellitus (T2DM) in pregnancy has a similar impact on infants as T1DM [4, 5], and PGDM has a greater negative impact on pregnancy outcomes compared to pregnancies complicated with GDM [2].

Despite the significant progress achieved in glycemic control in pregnant women with DM, pregnancy complications are still very common, especially in case of PGDM [6].

Short-term adverse outcomes can be divided into complications that occur: in utero (diabetic fetopathy, fetal macrosomia, intrauterine growth restriction, congenital malformations, antenatal fetal death); during labor (shoulder dystocia, birth injuries, intranatal death) and during the neonatal period (respiratory distress syndrome, metabolic, electrolytic and hematological disorders, hypertrophic cardiomyopathy, neonatal mortality). Long-term fetal outcomes include: overweight and obesity, impaired glucose tolerance or T2DM, metabolic syndrome with increased risk of cardiovascular disease and subtle neurophysiological dysfunctions.

Adverse fetal outcomes of a diabetic pregnancy can also be classified according to trimesters:

- First trimester – congenital malformations, fetal loss, intrauterine growth restriction;
- Second trimester – hypertrophic cardiomyopathy, erythremia, fetal loss, low intelligence coefficient;
- Third trimester – hypoglycemia, hypocalcaemia, hyperbilirubinemia, respiratory distress syndrome, macrosomia, hypomagnesaemia, intrauterine fetal death [48].

Adverse fetal outcomes of diabetic pregnancy

Diabetic Fetopathy (DF) is a complex and heterogeneous syndrome that develops in the fetus during the intrauterine period and is induced by genetic or acquired disorders of insulin secretion and / or peripheral cell resistance to insulin action and is characterized by specific phenotypic changes, congenital defects, significant metabolic and functional disorders of the newborn [7].

The definition of "diabetic fetopathy" is mainly used in Russian specialty literature and it is uncommon in Anglo-American literature, being partly replaced by the term of "diabetic macrosomia", with emphasis on birth complications such as shoulder dystocia, hypoxia and others, without counting the metabolic changes in this context [8].

The carbohydrate metabolism disorders during pregnancy contribute to the development of DF, which is most frequently characterized by macrosomia or intrauterine growth restriction (IUGR) and is one of the most serious and specific manifestations of maternal DM in the newborn, which increases the risk of birth trauma, perinatal morbidity and mortality [9].

The literature data on the frequency of DF is contradic-

tory. The incidence of DF ranges between 5.7% and 75.5% and depends on the type and degree of compensation of the maternal DM, the presence of vascular complications, associated obstetrical and extra-genital disorders, and the population that is subject to research. According to some authors, most newborns from mothers with T1DM (96%) and T2DM (85%) show signs of DF and only 49% of infants from mothers with GDM will develop these signs.[11].

Macrosomia. The concept of excessive fetal growth is expressed either by "macrosomia" or "large for gestational age" [12, 14, 15].

There is no general consensus on the definition of macrosomia or the underlying principles of diagnosis. Most recent studies and meta-analyses define macrosomia as birth weight ≥4,000 g. The American College of Obstetricians and Gynecologists recommends as a reference the weight of ≥4,500 g due to substantial increase of the rate of maternal, fetal and neonatal complications. Nevertheless, there is considerable variation in the definition of macrosomia (≥4,000 g, ≥4,100 g, ≥4,200 g, ≥4,500 g, ≥4,536 g regardless of gestational age, > 90th percentile, > 95th percentile or 2 deviations standard above the mean weight for corrected gestational age, gender and ethnicity) [12, 14, 15, 17, 23, 24].

Defining macrosomia with an absolute fixed birth weight has the advantage of making it easier to determine and remember, but does not take into account the influences of gestational age on the birth weight. Defining it as a newborn large for gestational age offers a potential solution for this problem. However, infants that are large for gestational age are also defined differently: infants with a body weight above the 90th percentile or the 95th percentile or greater than 2 standard deviations for the gestational age corrected for sex and ethnicity [6, 12, 18, 25].

Fetal macrosomia is the most common consequence of diabetic pregnancy, that usually becomes obvious from the 26-28th gestational week [26, 27] and its severity is mainly influenced by the maternal blood glucose level [14, 27]. Literature data on macrosomia frequency differs significantly by country and type of DM [16]. The prevalence of macrosomia in developed countries varies from 5% to 20% [15], and in developing countries - from 0.5% to 14.9% [14].

The prevalence of macrosomia studied in many countries of the world significantly varies from 1% (Taiwan) to 28% (Denmark), with the highest rates (20%) in Northern countries [16].

According to a recent study, the overall rate of macrosomia for the non-diabetic population is 7-9%, increasing to 20-45% in GDM [3]. Fetal macrosomia (birth weight ≥4,000 g) is found in 15-45% of newborns from mothers with GDM, 47% of mothers with T1DM compared to 12% of neonates from pregnant women without DM. In the last 2-3 decades, the incidence of macrosomia increased by 15-25% and is worldwide associated with increased rates of obesity and maternal DM [15, 19, 29, 30].

Fetal macrosomia can be estimated using clinical data (assessment of uterine height and maternal medical history), determination of insulin levels in the amniotic fluid, requiring an invasive procedure – amniocentesis, and ultrasound scans. Fetal ultrasound scan became an indispensable part of an obstetrical examination and is the elective method for the antenatal estimation of fetal weight [12]. Some studies demonstrated that the Ott, Hadlock IV and Coombs formulas are preferred for estimating fetal weight in fetuses <2,500 g and> 4,000 g. Formulas that combine all three parameters (bi-parietal diameter, femur length and abdomen circumference) provide the best estimation of fetal weight in terms of general accuracy and do not indicate a tendency to overestimate or underestimate real weight [12, 17, 23, 31].

There is evidence that serial biometric ultrasound scan throughout pregnancy, especially the evaluation of fetal abdomen circumference in the third trimester, can improve the predictive accuracy of fetal macrosomia in pregnant women with DM [24, 32, 33]. The majority of macrosomia prediction studies are based on ultrasound measurements of one parameter (abdominal circumference or subcutaneous tissue thickness) or combinations of measurements (abdominal circumference, biparietal diameter, femur length, and head circumference) to estimate fetal weight [12, 17, 23, 31]. Although the combined fetal biometric parameters or serial evaluation throughout pregnancy provide the best estimation of fetal weight, a recent meta-analysis revealed the utility, safety, and sufficiency of fetal abdominal circumference measurement only after 24 weeks of gestation for the estimation of newborns as large or small for gestational age [12].

Despite the fact that DM modifies the parameters of fetal biometry with the increase of the thoracic-abdominal parameter, there is still a lack of precision of the antenatal diagnosis of macrosomia using the ultrasound scan – insufficient method with a positive predictive value of only 65% for the detection of a fetus ≥4,000 g [16].

The effectiveness of three-dimensional ultrasound in the estimation of fetal weight is contradictory. Some authors have demonstrated that this method improves the estimation of fetal weight [12, 23, 24] and other studies have found the superiority of two-dimensional ultrasound performed 2 weeks before birth in predicting the birth weight of the newborn and fetal macrosomia in pregnant women with DM [24, 34].

Therefore, scientific literature confirms that the prediction of fetal macrosomia is complicated. Ultrasound scan was recognized as the most accurate method of estimating fetal weight. Unfortunately, the average error varies within a 300-550 g range, and ultrasound assessment of fetal weight adds some useful additional information to clinicians in estimating macrosomia [33].

Multiple conclusive clinical and experimental studies showed the association of PGDM (T1DM and T2DM) or GDM with macrosomia. DM remains a major cause of macrosomia despite improved obstetrical care [1, 12, 18]. Glycemic parameters of diabetic pregnant women in the third trimester are stronger predictors of fetal growth than blood

glucose levels in the I and II trimesters or in preconception period, the latter being more commonly associated with lower birth weight [35,36,37].

The first meta-analysis of epidemiological studies published in 2015, which explored the significance of GDM as an independent risk factor for macrosomia, included 5 cohort studies and 7 case-control studies. The authors found that GDM was associated with macrosomia independently from other risk factors (pre-gestational body mass index, pre-pregnancy obesity, pathological weight gain in pregnancy) [39]. Each type of DM is an independent risk factor for macrosomia [1, 12, 18], and effective treatment of hyperglycemia in pregnancy significantly reduces the rate of fetal macrosomia [14, 38].

According to Pedersen's hypothesis, the macrosomia observed in pregnant women with DM is a consequence of fetal hyperinsulinemia, a secondary condition of maternal hyperglycemia. Fetal hyperinsulinemia causes an increased use of cellular glucose, which contributes to hepatic glycogen deposit formation, decreases lipid mobilization and increases protein production. Insulin stimulates the incorporation of amino acids into proteins and in diabetic pregnancy it increases the assimilation of amino acids and protein synthesis and decreases protein catabolism. During the last 12 weeks of gestation, the fetus of a diabetic mother stores 50-60% more fat than the fetus of a mother without DM [19].

Fetal growth is the result of maternal hyperglycemia with increased trans-placental transfer of maternal glucose leading to fetal hyperglycemia (glucose being an important anabolic nutrient), the stimulation of insulin release by fetal β-pancreatic cells (hyperinsulinemia, insulin being an important anabolic hormone) and, as insulin is a major factor in fetal growth, it leads to macrosomia and other complications with an increased risk of developing obesity, T2DM and cardiovascular disease in adolescence or youth years. Pederson's hypothesis is fundamental for understanding the physiopathological consequences of DM during pregnancy. Subsequently, this theory has been modified to include the contributions of other nutrients in increased concentrations (amino acids, lipids, insulin growth factor) that may contribute to fetal hyperinsulinemia [3, 15, 19, 28, 51, 52].

Fetal hyperinsulinemia has the following effects:

- Excessive growth of insulin-sensitive tissues such as adipose tissue (especially around the chest, shoulders and abdomen), organomegalia (especially of the liver, spleen and heart) and accelerated maturation of the skeleton, which increases the risk of shoulder dystocia, perinatal mortality, birth trauma and the rate of cesarean section surgeries;
- Neonatal metabolic, electrolytic and hematological complications: hypoglycemia, hypocalcemia, hyperphosphatemia, hypomagnesemia, polycythemia, hyperbilirubinemia;
- In utero hypoxemia develops, which may increase the risk of antenatal mortality, polycythemia, hyperbilirubinemia and venous renal thrombosis of the fetus;
- Increased risk of long-term outcomes such as obesity and DM in childhood [6, 12].

Experimental and clinical studies found that fetal hyperinsulinemia is strongly associated with fetal macrosomia and increased adipose tissue. However, the high frequency of fetal macrosomia in diabetic pregnant women with adequate glycemia control and in pregnant women without DM confirms the involvement of factors other than maternal hyperglycemia and fetal hyperinsulinemia, in the development of fetal macrosomia [19].

While maternal hyperglycemia and fetal hyperinsulinemia are considered the main causes for excessive fetal growth, the exact aspects of the underlying mechanisms of macrosomia remain less clear. Not only poor glycemic control before and throughout pregnancy is a cause of fetal macrosomia but also hormonal, genetic, environmental, and constitutional factors, an angiopathy of utero-placental vessels with fetal subsequent hypoxia contributes to the development of fetal macrosomia. DM and poor glycemic control, pre-gestational obesity, pathological weight gain in pregnancy, a history of macrosomia, and parity are the main risk factors for macrosomia. Despite all these risk factors, many aspects of the weight at birth remain inexplicable [12, 40, 41, 42, 43].

For practical reasons, the causes of fetal macrosomia can be divided into non-modifiable factors (genetic factors, male gender, parity, age and maternal height) and modifiable maternal factors (pre-pregnancy body mass index, pathological weight gain, nutritional intake, the level of physical activity, smoking and metabolic parameters, especially DM and dyslipidemia) [32].

Although DM and maternal obesity are independently associated with pregnancy complications, the combination of T2DM or GDM with pre-gestational obesity has a greater effect on macrosomia and is associated with higher perinatal morbidity rates [6, 19].

Macrosomia, regardless of the cause, is associated with a higher risk for maternal complications (prolonged labor, birth assisted with forceps or vacuum, caesarean delivery, maternal trauma, postpartum hemorrhage) and neonatal complications: premature birth and complications associated with prematurity (respiratory distress syndrome, infection, jaundice, transfer to neonatal intensive care unit and perinatal mortality), birth injuries (shoulder dystocia, brachial plexus palsy, clavicle and humerus fractures), neonatal hypoglycemia, perinatal asphyxia, meconium aspiration, congenital abnormalities [12, 14, 18, 19, 20].

Therefore, fetal macrosomia is an obstetrical condition that affects an average of 10% of all pregnancies and may be associated with severe maternal, fetal and neonatal adverse outcomes. An early identification of risk factors (pre-gestational obesity, pathological weight gain, PGDM and GDM) allows applying the measures needed to prevent perinatal complications.

Intrauterine growth restriction (IUGR)

Infants with a birth weight below the 10th percentile are

considered small for gestational age. IUGR is more rarely associated with DM, and it occurs in about 20% of diabetic pregnancies, more frequently in pregnant women with T1DM with severe renal-vascular complications compared to a 10% incidence in infants born to mothers without DM. Maternal renal-vascular disorder is a common cause of the impairment of fetal growth in pregnancies complicated with DM. The newborns considered small for gestational age have an increased risk of low Apgar score at birth, respiratory distress syndrome, neonatal death, cardiovascular and metabolic disorders during the life [20].

Congenital malformations of neonates from diabetic pregnant women are the main cause of perinatal and infantile death. The risk of major congenital malformations in pregnancies complicated with DM is 2-5 times higher than in the general population, congenital abnormalities occur in 4-12% of cases [16,20], and in pregnant women with decompensated forms of DM the risk increases up to 20% [14, 20].

The most common congenital anomalies are the following [15, 26, 45, 46]:

- Cardiac: transposition of the great vessels, atrial septal defect or ventricular septum defect, aortic co-arctation, persistent truncus arteriosus, single ventricle;
- Of the central nervous system: anencephaly, microcephaly, encephalocele, meningomyelocele, holoprozencephaly, spina bifida;
- Skeletal: caudal regression syndrome (agenesia or hypoplasia of the sacral and coccidian bone, sometimes of lumbar vertebrae), femoral dysplasia;
- Renal: renal agenesis, hydronephrosis, duplicated ureter;
- Gastro-intestinal: duodenal atresia, anorectal atresia;
- Others: palatoschisis, microftalmia, intestinal atresia.

Newborns of mothers with PGDM are more likely to develop congenital malformations, with a similar incidence in both types of PGDM, and the development risk is highly associated with the length of DM before pregnancy [15, 20, 44, 45, 46].

Several authors reported an association between GDM and the same types of congenital malformations diagnosed in the descendants of women with PGDM, although some studies found a limited association of GDM with some congenital defects. The risk of congenital abnormalities in neonates of mothers with GDM is lower than the risk for neonates of mothers with PGDM [15, 44, 47]. The risk of congenital malformations does not significantly vary in women with T1DM and T2DM, being 1.9-10 times higher in the PGDM, 1.7-3 times higher in T1DM compared to the general population. In the case of GDM, the risk of congenital malformations is moderate and is slightly increased (1.1-1.3 times higher) compared to the general population, but is much lower than in women with PGDM and is probably also determined by cases of undiagnosed T2DM in patients with GDM [15, 44].In newborns of mothers with PGDM, the incidence of heart defects varies from 2 to 34 cases per 1,000 births, central nervous system abnormalities – from 1 to 5 cases per 1,000 births, musculoskeletal malformations – from 2 up to 20 cases per 1000 births, genital-urinary abnormalities – from 2 to 32 cases per 1,000 births and gastrointestinal defects – from 1 to 5 cases per 1,000 births [44, 49].

It is difficult to compare the frequency of congenital abnormalities associated with maternal DM due to the differences in diagnostic criteria of DM in different countries. It is also difficult to compare maternal DM rates among populations where the screening of the disease is not similar in all centers.

The results of several studies on the risk of congenital anomalies in neonates of mothers with GDM showed the 1.2-fold increase in congenital malformations for GDM compared to the general population. Pregnant women with GDM with a basal hyperglycemia > 120 mg / dl (> 6.7 mmol / l) or HbA1c ≥ 7.0% have a 3.4-fold higher risk, and for women with GDM with normal basal glucose there is no difference of risk compared to women without DM. It was found that there is a small, but statistically significant increase in the frequency of holoprosencephaly, bone abnormalities and genitourinary system malformations in children of mothers with GDM compared to non-diabetic pregnant women [44].

The pathogenesis of fetal malformations associated with PGDM is partially understood, but it is multi-factorial and correlates with several deficiencies of toxic nutrients or metabolites. Hyperglycemia, hypoxia, ketonemia, amino acid abnormalities and protein glycosylation were reported as potential teratogenic factors that may affect molecular signaling pathways with adverse effects on embryogenesis [15, 18, 20].

Embryo-developmental disorders during pregnancy complicated with DM were extensively explored in experimental and clinical studies. Available data indicate that there are many changes in the embryonic environment capable of inducing teratogenic development. The most important change is the increase in glucose concentration, which has a number of direct metabolic consequences on the embryo. However, there are other modifications with teratogenic effects - the excess of reactive oxygen molecules, elevated levels of ketone bodies and branched chain amino acids in the tissues of different fetal organs, but their mechanism of action still has to be elucidated. Likewise, newborn rats from mothers with chemically induced DM have an increased oxidative stress in different tissues, and there is an increase in reactive oxygen molecules and lipid peroxidation in the liver, kidney, brain and skin, and elevated levels of lipid peroxides in plasma [8, 14, 20].

Therefore, maternal DM, especially PGDM, has colossal consequences on the incidence of congenital anomalies [14, 16, 20].

Intrauterine fetal death. Approximately 50% of the cases of dead fetus births are related to uncontrolled maternal hyperglycemia, and the other cases are caused by fetal congenital infections or anomalies, placental insufficiency, maternal diseases. This increased risk is most commonly associated with T1DM, but it can also be encountered in other forms of DM. Compared with the general population, the risk of fetal mortality is 3-5 times higher in pregnant women

with T1DM, 2-3 times higher in women with T2DM, and pregnant women with GDM have a lower risk than women with PGDM. The birth of a dead fetus in pregnant women with DZG is more common at gestational age, suggesting that maternal hyperglycemia causes hyperinsulinemia and fetal lactic acidosis – the main causes of intrauterine death [13]. Antenatal fetal death in pregnant women with GDM is more common for gestational age fetus, suggesting that maternal hyperglycemia causes hyperinsulinemia and fetal lactic acidosis – the causes of intrauterine death [13]. Hypoxia and fetal heart failure, secondary to poor glycemic control, are probably the most important factors for antenatal mortality among pregnant women with DM [13].

Complications related to labor and delivery

Shoulder dystocia is a rare but serious obstetric complication that occurs in neonates with macrosomia and may lead to paralysis of the brachial plexus, fracture of the clavicle or of the humerus [15, 20]. In Denmark, the risk of shoulder dystocia among vaginal births is 6% at women with T1DM. A quarter of these neonates need resuscitation at birth, and some suffer from lesions of the bones and nerves. The incidence of brachial plexus paralysis and fractures of neonates born alive from mothers with PGDM is 10 times higher than in the general population [8, 20].

Preterm birth, being one of the major causes of fetal death, is found in about 10% of the pregnancies, in 50% of women with DM and is 4.8 times higher in pregnant women with DM than in the general population [21]. Patients with T1DM have an increased risk of premature birth. Recent cohort studies demonstrated that premature birth rates were 24-33.9% in pregnant women with T1DM, and previous studies reported values ranging from 26.2% to 31.1% [22].

Cesarean section. Women with DM generally have higher cesarean section rates. The frequency of this procedure in pregnant women with DM worldwide is about 42.7-78%, compared to a much lower rate in the general population (20%). The number of cesarean sections does not significantly vary in pregnant women with T1DM and T2DM. A number of DM-induced factors (maternal obesity, fetal macrosomia, polyhydramnios and diabetic microvascular complications) are also associated with an increased risk of surgery. Simultaneously with the protection of the newborn from hypoxic-ischemic cerebral lesions and the complications related to macrosomia by avoiding vaginal birth, the potential side effects of a cesarean surgery are delayed or discontinued breastfeeding and respiratory morbidity (transient tachypnea of the newborn or surfactant deficiency). These complications frequently lead to the admission of the newborn to the neonatal intensive care unit or resuscitation unit and separation of the mother from the child [21, 22].

Neonatal adverse outcomes

Respiratory distress syndrome. Neonates from mothers with DM have an increased risk of respiratory disorders. The incidence of respiratory distress syndrome is 5-6 times higher for any gestational age compared to non-diabetic pregnancies. Respiratory distress syndrome is typical in newborns with DF, with a frequency of 13-40% in neonates of mothers with PGDM and up to 5% in neonates of mothers with GDM [3, 8, 14, 15, 18, 20].

The pathogenesis of respiratory distress syndrome in neonates from mothers with DM is poorly understood, but several theories are possible. First, hyperinsulinemia inhibits the synthesis and secretion of surfactant by type 2 pneumocytes with a delayed pulmonary maturation. Secondly, these children are often prematurely born with surfactant deficiency. Therefore, there is a direct adverse impact of hyperglycemia on the fetal metabolism of lung surfactant. Third, cesarean delivery due to macrosomia increases the risk of transient tachypnea in the newborn, while polycythemia predisposes the neonate to persistent lung hypertension. Finally, the cause of respiratory distress syndrome may also be meconium aspiration syndrome and hypertrophic cardiomyopathy. Nevertheless, respiratory distress syndrome affects newborns in pregnancies with severe PGDM. Frequency and risk of respiratory distress syndrome in GDM cannot be accurately determined due to insufficient data [3, 7, 8, 14, 15, 18, 20].

Metabolic, electrolytic and hematological neonatal disorders are associated with fetal hyperinsulinemia [18].

Hypoglycemia is the most common metabolic complication secondary to fetal hyperinsulinemia with a prevalence ranging from 25% to 76% depending on the definition of hypoglycemia threshold and maternal glycemic control at birth. However, in the vast majority of cases it is biochemical hypoglycemia, i.e. asymptomatic neonatal hypoglycemia. Pregnant women with the highest basal glucose level have infants with the highest frequency of neonatal clinical hypoglycemia – 5-7%. The risk of hypoglycemia is the highest in large for gestational age infants and premature newborns [3, 15, 18, 25, 50].

A recent study defined capillary blood glucose levels as normal (≥2.5 mmol / l), mild hypoglycemia (2.2-2.4 mmol / l), moderate hypoglycemia (1.6-2.1 mmol / l) and severe hypoglycemia (<1.6 mmol / l). Among newborns from pregnant women with GDM, the prevalence of hypoglycemia was 25%: 12.1% had mild hypoglycemia, 10.5% moderate hypoglycemia and only 2.6% severe hypoglycemia [50].

Hypocalcaemia is detected at a calcium concentration of <2 mmol / l (<7 mg / dl) or ionized calcium concentration that is <1.1 mmol / l (<4 mg / dl) [14]. These complications occur up to 50% of cases, being usually associated with hyperphosphataemia and occasionally with hypomagnesaemia, all of which rarely have clinical significance. The etiology of neonatal hypocalcaemia is unclear, but severe DM and neonatal hypoparathyroidism may be possible causes [3, 18, 20].

Polycythemia (a hematocrit that is > 65%) occurs in 13-33% of cases [14, 18, 20], is more commonly determined in neonates from mothers with DM. Relative cell hypoxia determines an increased erythropoietin secretion, which in return increases fetal erythrocyte production. Neonatal polycythemia can cause excessive neonatal jaundice by red

cell lysis and blood clotting syndrome with complications caused by vascular stasis [3, 18, 20]. Hyperbilirubinaemia occurs in 11-29% of cases [14, 15, 18, 20].

Hypertrophic cardiomyopathy

DM in pregnant women affects the fetal heart structurally (cardiac malformations, hypertrophic cardiomyopathy) and functionally (even in the absence of structural changes) with long-term consequences. Fetal hyperinsulinemia, as a result of abnormal maternal glycemic control, causes hyperplasia and hypertrophy of the fetal myocardium with the development of hypertrophic cardiomyopathy - interventricular septal hypertrophy and, to a lesser extent, ventricular hypertrophy with left ventricular outflow tract obstruction. However, clinical experience shows that even infants of DM women with adequate glycemic control may have septal hypertrophy. Therefore, other maternal major risk factors that affect the fetal heart were identified, such as hypertriglyceridemia, obesity, increased oxidative stress and placental factors [8, 14, 20].

Hypertrophic cardiomyopathy occurs in 25-35% of infants born to mothers with DM and sometimes it leads to significant morbidity and mortality, depending on the severity and extent of cardiac hypertrophy and aortic obstruction. Fortunately, most cases of hypertrophic cardiomyopathy are transient and asymptomatic, do not require treatment and have a spontaneous echocardiographic resolution in the first months after birth [8, 14, 20].

Perinatal mortality. Until the discovery of insulin, pregnancies in women with DM were often associated with perinatal mortality. The perinatal mortality rate was around 65%, and maternal mortality was up to 30%. Insulin reduced maternal mortality by reducing the frequency of diabetic ketoacidosis, improving the fertility of women with DM, but perinatal mortality, although reduced, remained high [13,22].

Perinatal mortality in pregnant women with T1DM is 5 times higher, with PGDM - 4 times higher and neonatal mortality in pregnant women with DM of any type – 15 times higher compared to the general population [18, 20, 21]. One of the largest recent population-based studies revealed that perinatal mortality is 3 times higher and infant mortality 9 times higher in pregnant women with PGDM compared to those without DM. Major congenital abnormalities, maternal hypertension, and premature birth are important factors in increasing the mortality of children born of diabetic mothers. Overall, the perinatal mortality rate is almost identical for mothers with T1DM and T2DM [18].

Studies with fetal blood sampling confirm that hyperglycemia is associated with fetal hypoxia and acidosis. In all types of DM there are additional risk factors associated with perinatal mortality – diabetic angiopathy, hypertension and IUGR. In T2DM and GDM, compared to pregnant women without DM, there is a so-called "triad" of factors for intrauterine death – the relatively greater age of pregnant women, higher incidence of obesity and high blood pressure [13]. Although several factors may influence the perinatal mortality rate, the blood glucose threshold <6.1 mmol / l (<110 mg / dl) is possibly a major factor in preventing this complication. However, unlike PGDM, the increase in the fetal death rate in the 2nd and 3rd trimesters of pregnancy is questionable in GDM and can be attributed to previously undiagnosed T2DM [14].

A systematic review of the literature and meta-analysis of 33 observational studies, published in 2009, that compared maternal and fetal outcomes in pregnant women with T1DM and T2DM, found that pregnant women with T2DM had a lower level of HbA1c at the first OB visit, but a higher incidence of perinatal mortality, with no significant differences in major congenital malformations, antenatal, and neonatal mortality. Therefore, despite lower glycemic disorders, women with T2DM, compared to women with T1DM, did not show better perinatal outcomes [10].

The complications described above are short-term adverse outcomes, but long-term consequences may also occur. Children born from pregnancies complicated with GDM are at a higher risk of developing obesity, glucose intolerance and DM in adolescence and early adulthood. The lifetime risk to develop T1DM for children of diabetic mothers is 1.3%, and for children of diabetic fathers is 5.7%, while the risk of developing T1DM is much higher - about 50% [18].

Conclusions

1. Maternal diabetes, predominantly PGDM, has a significant impact on the incidence of both short-and-long-term complications in the fetus and newborn.

2. Short-term adverse outcomes include fetal complications (diabetic fetopathy, macrosomia, IUGR, congenital malformations, fetal antenatal death), complications related to labor and delivery (shoulder dystocia, birth injuries, intranatal death) and neonatal adverse outcomes (respiratory distress syndrome, metabolic, electrolytic and hematological neonatal disorders, hypertrophic cardiomyopathy, perinatal mortality).

3. Long-term fetal outcomes include overweight and obesity, impaired glucose tolerance or

T2DM, metabolic syndromes with an increased risk of cardiovascular disease and subtle neurophysiologic dysfunction.

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BOOK REVIEW

Text book "Rehabilitation in patients with the diseases of internal organs, of muscular and skeletal systems and of connective tissue"

Printing company "Totex-Lux", Chisinau, 2017, 412 pages

The authors: Vasilii Andreev, MD, PhD, Professor Emeritus, Participant in the Second World War as a soldier and first-aid man in the rifle battalion, artillery battery of Leningrad and second Ukrainian fronts;
Ion Tabirna, MD, PhD, Professor Consultant; **Ghenadie Bezu,** MD, PhD, Associate Professor
Department of Internal Medicine and Semiology
Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova

The book considers at a contemporary scientific level, the problems of etiology, pathogenesis, classification and the clinical picture with types and stages of development, complications, outcomes and also diagnosis including the differential one – all these in the diseases of internal organs, of the muscular and skeletal systems and of the connective tissue.

The book is 412 pages long and is composed of preface, abstract in English and five chapters, which reveal problems related to diseases of internal organs, of muscular and skeletal system and of connective tissues.

The distinctive feature of this book is that it provides basic information about diseases of internal organs, of muscular and skeletal systems and of connective tissues along with determining the degree of limited potentialities and working capacities with further rehabilitation in many nosological conditions.

The material exposed in the book will help clinicians, general practitioners and other categories of doctors to direct patients to the Medical Council in order to assess the degree of the impairment of potentialities and working capacities.

This is the first book with such a complex content which treats issues of the impairment of potentialities and working capacities after the acknowledgement of the Government Decree (2013) regarding the new statute of the assessment of patients with health problems mentioned above.

The book is written in an accessible language and is easy for reading. I would like to congratulate the authors with the publishing of such a useful book, which will become indispensable for many physicians from our country.

In fact this is a textbook classified as a brief presentation of diseases of internal organs and other conditions. I recommend translating and publishing it into Romanian and English for further distribution to all medical institutions from the Republic of Moldova and abroad.

The textbook is dedicated to the 70th anniversary of Nicolae Testemitsanu State University of Medicine and Pharmacy of the Republic of Moldova and to the 90th anniversary of the birth of the academician Nicolae Testemitsanu.

Nicolae Bodrug, MD, PhD, Professor
Chairman of the Department of Occupational Diseases
Nicolae Testemitsanu State University of Medicine and Pharmacy Chisinau, the Republic of Moldova

Monograph "Primary Hypothyroidism (clinical, pathogenic, diagnostic and therapeutic aspects)"

Printing company "Balacron Editions", Chisinau, 2017, 191 pages

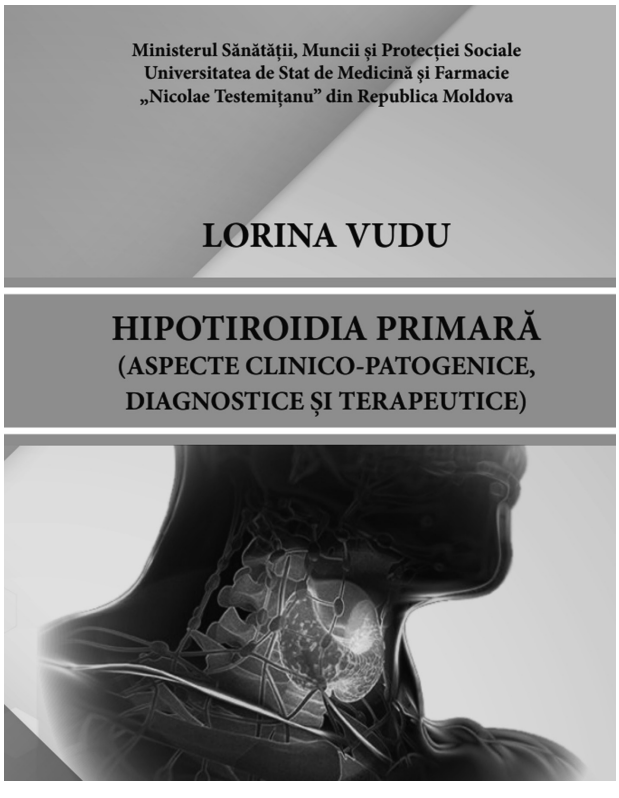
The author: Lorina Vudu, MD, PhD, Associate Professor
Department of Endocrinology, Nicolae Testemitsanu State University of Medicine and Pharmacy
Chisinau, the Republic of Moldova

The monograph "Primary Hypothyroidism (clinical, pathogenic, diagnostic and therapeutic aspects)" is devoted to an important problem in clinical endocrinology in the Republic of Moldova and general medicine. The number of people with autoimmune thyroid disease has considerably increased, in adults and children, after the Chernobyl nuclear explosion, which has led to an increase in the number of people with hypothyroidism. Hypothyroidism is one of the most widespread endocrine diseases with a prevalence of about 2% in the general population, subclinical hypothyroidism, which usually evolves to manifest hypothyroidism, has a prevalence of up to 10%, and high levels of antibodies to thyroperoxidase (indicators of autoimmune process), which is a predictive factor of autoimmune hypothyroidism of up to 11%.

Both current literature review, which refers to hypothyroidism, as well as own data are analysed in the monograph. The monograph contains more than 500 bibliographic sources.

The first chapter of the monograph is dedicated to the incidence, classification of hypothyroidism, and regulation of function of the thyroid gland. Close attention is paid to au-

toimmune thyroiditis, which is one of the commonest causes of primary hypothyroidism and which has an increasing incidence and prevalence in the Republic of Moldova. This chapter presents the latest data on the role of T and B lymphocytes in the development of the disease. Evidence is presented about the pathogenetic mechanisms that cause Hashimoto's thyroiditis: molecular mimicry and expression of HLA system antigens on thyrocytes, activation of thyroid cells apoptosis through a Fas-ligand interaction. Confirmations of genetic susceptibility are presented and the precipitating factors of autoimmune thyroiditis, as well as the effect of thyroid hormones, the mechanisms of regulation of thyroid function are described in the monograph.



The chapter "Endocrine and metabolic disorders in hypothyroidism" presents the natural continuation of the first chapter, which describes the metabolic disorders occurring in hypothyroidism. Changes in lipid metabolism are best studied. Decreasing thyroid function not only increases the number of LDL particles, but also promotes LDL oxidation. Abnormalities of lipid metabolism associated with hypothyroidism predispose to the development of atherosclerotic coronary artery disease. Emphasis is placed on the development of fatty liver disease in hypothyroidism, which may be one of the explanations of metabolic disorders. Decreased thyroid function causes changes in glucidic metabolism. Regional cerebral disruptions of glucose metabolism can cause alteration of superior mental functions. The anabolic effect of thyroid hormones in physiological doses in protein metabolism is well known, but the pathological changes caused by the impairment of this metabolism in hypothyroidism are not sufficiently related in modern literature. The author emphasizes that there are insufficient studies of the mechanisms of protein and glucidic metabolism disorders, although disruptions of these metabolic processes underlie the development of various signs and symptoms of hypothyroidism.

A separate chapter is devoted to the results of the author's own research on changes in the profile and content of free blood amino acids in patients with primary autoimmune hypothyroidism, which have not yet been studied sufficiently. These studies are of particular interest because, according to contemporary data, the main role of thyroid hormones is to ensure the expression of many genes, which determines the significance of free amino acids metabolism research, as indicators of protein metabolism. The author has identified the modifications of both spectrum and content of amino acids in part, as well as of functional groups – immunogenic, ketogenic, glycogenic, proteinogenic. Most of the data is new.

Chapter 4 summarizes the current knowledge of national and international literature on clinical picture, vegetative disorders in hypothyroidism. The importance of this chapter is that it is taken into account that hypothyroidism, being a polyorganic disorder, determines the polymorphic picture. The above mentioned testifies about the need for special attention from physicians of various specialties towards patients with low thyroid function in order to diagnose them early.

In the 5th chapter dedicated to the neuropsychological and cognitive disorders, the author describes their variability – both decrease of psycho-emotional and mental processes, disorder of mnestic processes, as well as a general intellectual decline, depressive and paranoid manifestations. The attention to these disturbances is determined by the fact that substitution treatment only is not sufficient in treating patients with hypothyroidism. In the chapter are described the methods applied to detect cognitive impairment – P300 cognitive evoked potentials and changes of P300 potential in people with hypothyroidism. Another point of interest is data regarding the pathogenetic mechanisms of correlation between cognitive indices and vegetative changes. Literature data analysis shows that most people with hypothyroidism have psycho-emotional and cognitive disorders, but the interrelation of the thyroid system with superior mental functions is not clear.

In chapter 6 is discussed the treatment of hypothyroidism. The author mentions that substitution treatment in most cases reverses pathological symptoms, but normalization of cognitive and emotional-motivational processes is not always achieved, requiring additional approach of some pathological manifestations in hypothyroidism.

In conclusion, the monograph of Mrs. Vudu Lorina is a premiere for the endocrinology specialty in the Republic of Moldova through the high-relevance approached field. The monograph "Primary hypothyroidism (clinical, pathogenic, diagnostic and therapeutic aspects)" is a fundamental work of major scientific value and practical significance. It is recommended to endocrinologists, residents, students, lecturers and practitioners in other fields.

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