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Nicolae Testemitsanu State University of Medicine and Pharmacy

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al Republicii Moldova.
Universitatea de Stat de Medicină și
Farmacie „Nicolae Testemițanu”

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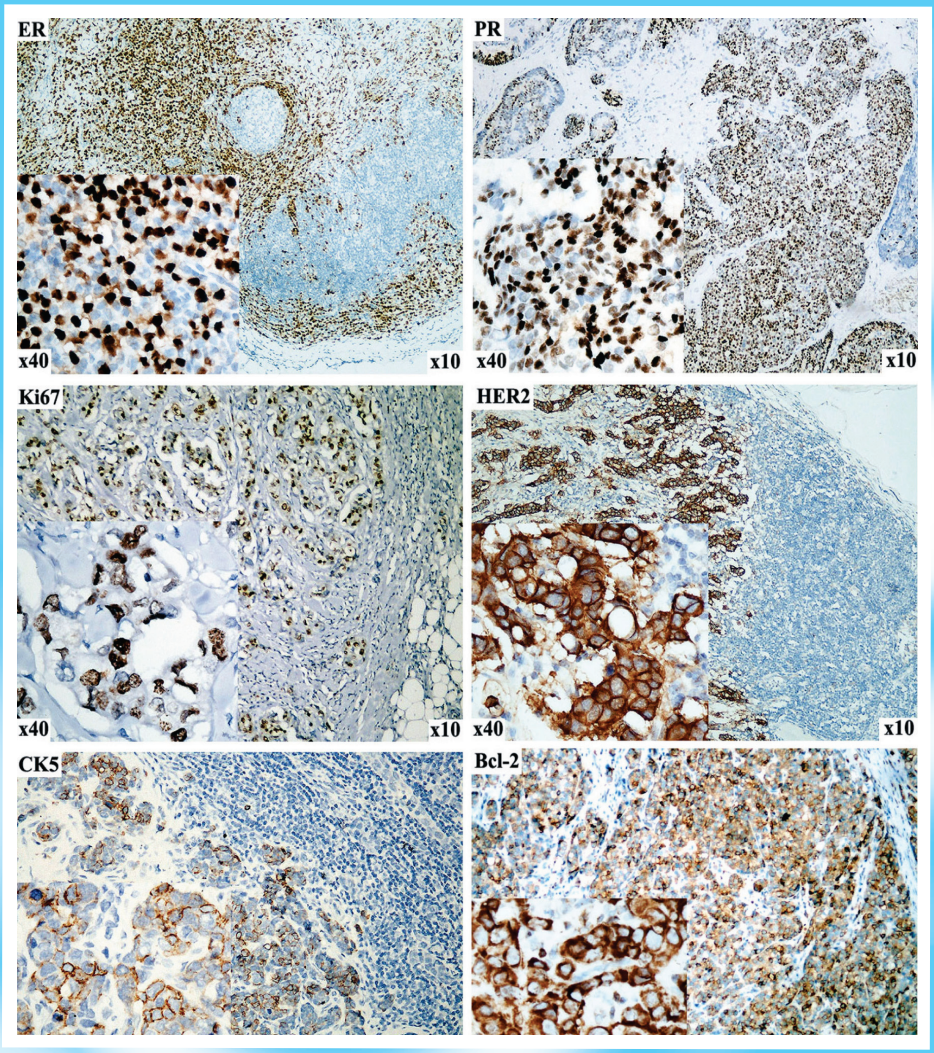

Representative images of ER, PR, HER2, CK5, Ki67, BCL2 immunohistochemical staining in primary breast carcinoma and corresponding lymphonodal metastases.

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Welcome to the scientific and medical journal Curierul Medical!

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

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

Bine ați venit la revista științifică medicală Curierul Medical!

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

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

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

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RESEARCH STUDIES

B-cell lymphoma-2 receptor in human primary breast and its lymph node metastases: more than a surrogate marker

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Abstract

Background: Due to its anti-apoptotic and anti-proliferative contradictory functions, BCL2 role in breast carcinoma progression is not clearly understood. The purpose of this study was to highlight BCL2 expression during metastatic progression of invasive breast carcinoma of no special type (NST).
Materials and methods: The specimens, primary tumors and corresponding lymph node metastases (LNM) from 84 patients were immunostained for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor (HER)-2, basal cytokeratin CK5, nuclear protein Ki67 and B-cell lymphoma (Bcl)-2 receptor.
Results: BCL2 expression was higher at primary site than in axillary metastases. Its score correlates positively with hormone receptors' level and negatively with HER2, CK5 and Ki67 at both sites. Switch of molecular profile was determined in 22.62% of cases. BCL2 expression was not influenced by subtypes switch. Changes of BCL2 expression were found in 25% of cases with stable molecular subtype. The Luminal A and Luminal B/Ki67 were encountered in the majority of BCL2 transitions, mainly from positive to negative state.
Conclusions: Molecular subtypes and BCL2 expression are not stable during tumor progression and metastatic development. In the present study we established immunohistochemically that BCL2 is not influenced by subtypes' transitions. BCL2 switches were encountered only in cases with a stable HER2, Luminal A or B phenotypes. We expect a further confirmation of our results by other research groups.
Key words: BCL2, breast carcinoma, immunohistochemistry, molecular subtypes, metastases.

Introduction

Breast carcinoma is the most common cause of death among women, and despite all efforts the drivers of this malignancy are not completely elucidated. Cancer occurs as the result of a disturbance in the balance between cell growth and cell death. Over-expression of anti-apoptotic genes or under-expression of pro-apoptotic genes can result in the lack of cell death, mechanisms that have been demonstrated in breast cancer too. Besides well-known prognostic factors, such as tumor size, histological type and grade, vascular invasion, identifying of new molecular factors has become the objective of many research studies. One of these potential markers is B-cell lymphoma (Bcl)-2 receptor.

BCL2 is a member of regulator proteins that regulate cell death, by either acting as pro-apoptotic or anti-apoptotic [1, 2]. BCL2 is specifically considered as an important anti-apoptotic protein and is thus classified as an oncogene. This protein, apart from its well-known inhibition of apoptosis, can also inhibit progression of the cell cycle by delaying entry into the S phase and maintaining cells in the G0 phase.

BCL2 in addition to HER2 and p53 is considered a tumor-related protein that has the potential to further improve individualization of patient management, by predicting response to chemotherapy, hormone therapy and radiotherapy [3]. Due to its anti-apoptotic function BCL2 is considered an important factor in the modulation of hormonal/anti-hormonal responsiveness exhibited by tumors [4]. Patients with elevated BCL2 immunostaining appeared to have the greatest benefit from endocrine therapy. In addition, BCL2 overexpression is associated with favorable outcome and its effect in relationship to estrogen receptor (ER) status. Some data suggest that BCL2 expression is a predictive factor for response to chemotherapy particularly in anthracycline-based chemotherapy [5]. Other report showed this marker has no significant value for the therapeutic strategy [6]. Contrary, clinicopathological data suggests that BCL2 expression correlates with aggressive, prometastatic behavior in breast cancer [7].

In the last decade, the gene analysis led to identification of molecular subtypes and defined the gene-expression prognostic signatures [8, 9]. By analysis of protein expression using immunohistochemistry it has been identified that molecular subtypes characterized with surrogate markers are similar to those derived from gene expression arrays [10, 11]. Nowadays, it seems to be important to investigate the molecular profile of metastases too. Recent data reveal the instability of receptors throughout the metastatic process [12, 13]. This supports the hypothesis that the malignant phenotype is not pre-determined, but continues to evolve throughout its natural history. In comparison to basic, five

commonly accepted markers in breast cancer stratification (ER, PR, HER2, CK5, Ki67), nowadays is not clear the future of BCL2 positive tumor cells in the lymphonodal environment. The aim of the current study was to compare BCL2 expression from primary tumor with corresponding lymph node metastases (LNM) in association with hormone receptors (estrogen (ER), progesterone (PR)), HER2 (human epidermal growth factor receptor-2), basal cytokeratin CK5 status and molecular subtypes.

Material and methods

Patients. In this retrospective study there were analyzed specimens (breast carcinoma of no special type and corresponding axillary lymph node metastases) from 84 patients of 33-86 years old. In all cases patients underwent radical mastectomy and lymph nodes dissection, without prior chemo- and radiotherapy. Histopathological diagnosis was assessed by two pathologists and cases suitable for immunohistochemistry were carefully selected.

Ethical issues. This study was based on patients' informed consent and approved by the Ethics Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy from Chisinau, the Republic of Moldova (approval number 21/13/31.03.2014).

Specimen processing and immunohistochemistry. The specimens were fixed in 10% phosphate buffered formalin for 24-48h and paraffin embedded (Paraplast High Melt, Leica Biosystems). Primary tumor and its LNM were placed in one block and 5-µm thick step sections were cut. The immunohistochemical assessment included 6 surrogate markers (from Leica Biosystems, except HER2), for ER (clone ER/6F11), PR (clone Pr16), human epidermal growth factor receptor 2 (HER2/polyclonal/ DakoCytomation), marker of proliferation Ki67 (clone K2), basal cytokeratin CK5 (clone XM26) and BCL2 (clone BCL2/100/D5). Incubation with primary antibodies was followed by the use of HercepTest PharmDx Kit (DakoCytomation) and Bond Polymer Refine Detection System (Leica Biosystems, Newcastle Upon Tyne, UK). All cases were evaluated also by FISH as international rules recommend (PathVysion HER-2 DNA Probe Kit II, Abbot). Slides were processed automatically on Leica Bond-Max autostainer (Leica Microsystems GmbH, Wetzlar, Ger-

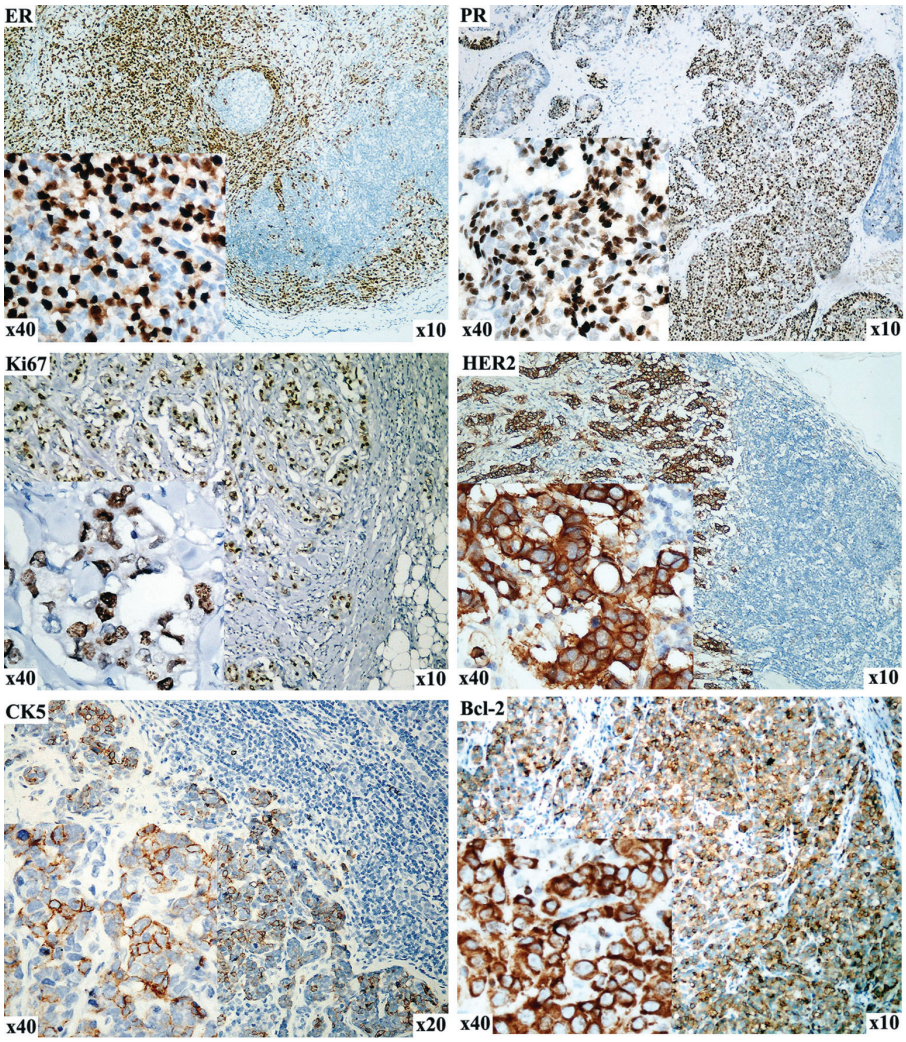

Fig. 1. Representative images of ER, PR, HER2, CK5, Ki67, BCL2 immunohistochemical staining in primary breast carcinoma and corresponding lymphonodal metastases (color version look on the front page of the cover).

many). The hematoxylin solution, Harris modified (HHS32, SigmaAldrich) was used for counterstaining (fig.1).

Microscopic evaluation. The hormone receptors were scored as the percentage of nuclear positively stained cells from at least 1000 cells assessed. We followed the guidelines of ER and PR assessment purposed by Allred et al. [14]. Cases scored +1 – +3 were considered positive. The threshold of positivity was 10%.

The HER2 assessments were done on LeicaBond Oracle HER2 IHC System (LeicaBiosystem). The HER2 status was interpreted in accordance with American Society of Clinical Oncology recommendations [15]. Cases interpreted as +2 and +3 were considered positive. Leica HER2 control slides ensured the control and accuracy of our decisions.

We used a 14% threshold for Ki67. This marker, as well as hormone receptors were counted using a semi-quantitative method performed by Suciu et al. [16].

The basal cytokeratin CK5 was interpreted in Azoulay's et al. manner [17]. Cases evaluated as +1 – +3 were considered positive.

The BCL2 evaluation was based on Callagy et al. recommendations: 0 – no staining; +1 – until 10% of cells show cytoplasmic pattern; +2 – 10-50%; +3 – more than 50% [18]. Cases scored as +2 and +3 were considered positive.

Based on Goldhirsch et al. recommendation we clustered molecular subtypes, as follows: ER^+ and/or PR^+, $HER2^-$, $CK5^-$, Ki67<14% as Luminal A; ER^+ and/or PR^+, $HER2^+$, $CK5^-$ as Luminal B/HER2; ER^+ and/or PR^+, $HER2^-$, $CK5^-$, Ki67>14% as Luminal B/Ki67; ER^+ and/or PR^+, $HER2^+$, $CK5^-$, Ki67>14% as Luminal B/HER2/Ki67; ER^-, PR^-, $HER2^+$, $CK5^-$ as HER2-overexpressed; ER^-, PR^-, $HER2^-$ and $CK5^+$ as Basal-like; ER^-, PR^-, $HER2^-$ and $CK5^-$ as unclassified [19].

Image acquisition and statistical analysis. A Nikon Eclipse 80i microscope with Nikon DS-Fi1 installed camera and Nis-elements BR 2.30 imaging software were used for microscopic evaluation (Nikon Instruments Europe BV). For descriptive statistics and Pearson's correlation assessment WinStat 2012.1 software was used (R. Fitch Software, Bad Krozingen, Germany). For all the tests a value of p£0.05 was considered significant. In order to determine the shifting direction of subtypes (from basal to Luminal and *vice versa*) unclassified subtype was assigned as "1", Basal-like as "2", $HER2^+$ as "3, Luminal B/HER2-"4", Luminal B/HER2/Ki67 –"5", Luminal B/Ki67 –"6" and Luminal A was equated with "7".

Results

Histological assessment revealed that the most frequent histological grade is G2, determined in 45 cases (53.6%), the share of G1 constitutes 6%/5 cases and G3 respectively in 40.5%/34 cases. In relation to BCL2, the majority of positive scores were determined in cases with G2 (36 cases/42.9%) and G3 grades (23 cases/27.4%) In primary tumor the surrogate markers for hormone receptors had the highest rate. BCL2 marker was positive in 62 cases/73.8% (tab. 1).

Table 1

Surrogate markers positivity in primary tu

Score	ER		PR		HER2		CK5		BCL2	
	No	%	No	%	No	%	No	%	No	%
0	16	**19.0**	26	**31.0**	62	**73.8**	73	**86.9**	17	**20.2**
1	3	**3.6**	5	**6.0**	4	**4.8**	5	**6.0**	5	**6.0**
2	9	**10.7**	13	**15.5**	5	**6.0**	4	**4.8**	9	**10.7**
3	56	**66.7**	40	**47.6**	13	**15.5**	2	**2.4**	53	**63.1**
	84 cases/**100%**									

In relation to hormonal receptors the positive BCL2 highest score was determined in cases with high expression of ER and PR receptors (tab. 2). And *vice versa* increasing of the HER2 and CK5 scores leads to decreasing of BCL2 score.

The proliferation marker Ki67 was considered positive (≥14) in 50 cases/59.52% of primary tumors. A positive BCL2 was accompanied with positive Ki67 (≥14) in 32 cases/38.10% and in 30 cases/35.71% with a Ki67<14. In 18 cases/21.43% with high Ki67 level, BCL2 was considered negative.

The most frequent subtype at primary site was Luminal B (45 cases/53.6%), followed by Luminal A (26 cases/31%) and hormone-negative group (13 cases/15.5%). By comparing the molecular profile and BCL2 score the highest rate of positivity was determined in Luminal B (39.29%) and Luminal A subtype (28.57%). The ratio of positive/negative BCL2 marker in HER2 overexpressing subtype was 50/50 – (4.76% positive)/(4.76% negative). Appropriate ratio was determined for Luminal B/HER2/Ki67 – 4.76% positive/3.57% negative.

By comparing molecular profile of primary tumor and its LNM we determined a transition of subtype to another one in 19 cases/22.62%. We have to mention that BCL2 expression was stable in spite of these switches. Transitions of BCL2 score (21 cases/25%) we found in the group, where molecular subtype at both sites is similar (tab. 3).

The highest percentage of BCL2 transition was encountered in Luminal A (8 cases/9.52%) and Luminal B/Ki67 (8 cases/9.52%). HER2-positive cases (4 cases/4.76%) associated in primary tumor with positive BCL2, preserved molecular subtype in metastases, but became BCL2 negative. In the same manner, from positive BCL2 to negative, proceed Luminal B/Ki67 and Luminal A, in 7 cases/8.33% each. The main intention of BCL2 expression throughout metastatic process was rather loss than acquiring – in 19 cases (from 21 changed), direction was from positive in primary tumor to negative in lymph node metastases. This is visible and by comparing the number of BCL2 positive cases at both sites – 62 cases/73.81% in primary tumor and 44 cases/52.38% in LNM.

BCL2 expression was analyzed in relation to proliferation activity. Tumors were grouped in 2 categories in accordance with Ki67 level: low proliferating (<14) and high proliferating (≥14). In 7 cases of Luminal A, where BCL2 changed during progression from negative to positive, the Ki67 had a low level

Table 2

BCL2 expression in relation to ER, PR, HER2, CK5 surrogate markers score at primary tumor site

BCL2 score	No	%	No	%	No	%	No	%	Total	
	ER score									
	0		1		2		3		No	%
0	10	11.9			3	3.6	4	4.8	17	20.2
1					1	1.2	4	4.8	5	6
2	2	2.4	1	1.2	1	1.2	5	6.0	9	10.7
3	4	4.8	2	2.4	4	4.8	43	51.2	53	63.1
Total	16	19.05	3	3.57	9	10.71	56	66.67	84	100
PR score										
BCL2 score	No	%	No	%	No	%	No	%	No	%
	0		1		2		3			
0	10	11.9	3	3.6	2	2.4	2	2.4	17	20.2
1					2	2.4	3	3.6	5	6
2	3	3.6			2	2.4	4	4.8	9	10.7
3	13	15.5	2	2.4	7	8.3	31	36.9	53	63.1
Total	26	31.0	5	6.0	13	15.5	40	47.6	84	100
HER2 score										
BCL2 score	No	%	No	%	No	%	No	%	No	%
	0		1		2		3			
0	9	10.7			1	1.2	7	8.3	17	20.2
1	4	4.8	1	1.2					5	6
2	7	8.3					2	2.4	9	10.7
3	42	50.0	3	3.6	4	4.8	4	4.8	53	63.1
Total	62	73.8	4	4.8	5	6.0	13	15.5	84	100
CK5 score										
BCL2 score	No	%	No	%	No	%	No	%	No	%
	0		1		2		3			
0	10	11.9	4	4.8	1	1.2	2	2.4	17	20.24
1	5	6.0							5	6
2	8	9.5	1	1.2					9	10.7
3	50	59.5			3	3.6			53	63.1
Total	73	86.9	5	6.0	4	4.8	2	2.4	84	100

(<14). Other 7 cases of Luminal B/Ki67, where BCL2 from positive changed to negative, Ki67 preserved a high rate at both sites. If in Luminal subtypes the proliferation marker preserves similar quotes at both sites, in HER2-overexpressed it changed randomly. In the last 7 cases some correlation of Ki67 level with BCL2 switch was not observed.

The statistical assays revealed significant, positive correlation of BCL2 level with type of molecular profile, level of ER, PR markers and negative correlation with HER2, CK5 and Ki67.

Discussion

Breast carcinoma is the most common cause of death among women. Despite implementation of screening programs the incidence of this tumor worldwide is still increasing. Besides well-known prognostic factors, such as tumor size, histological type and grade, vascular invasion, identifying of new molecular factors became the objective of many research studies. Failure to undergo apoptosis is considered a major mechanism of cancerogenesis and chemoresistance.

A marker which is involved in inhibition of apoptosis is B-cell lymphoma (Bcl)-2 receptor. It has been shown to contribute to oncogenesis because it can transform and immortalize cells in cooperation with c-myc, ras or viral genes. Del Bufalo et al. consider that BCL2 overexpression enhances both tumorigenicity and metastatic potential of tumor cells by inducing metastasis-associated properties [20]. Rochaix et al. found that BCL2 expression in tumors

Table 3

Molecular subtypes and BCL2 evolution in tumor progression: a comparative study of primary tumor and corresponding metastases

Molecular subtype		BCL2 expression		No. cases	%	
Tm	Mt	Tm	Mt			
Unclassified	Unclassified	-	-	2	2.38	
Unclassified	Unclassified	+	+	1	1.19	
Basal-like	Basal-like	-	-	1	1.19	
HER2	HER2	-	-	3	3.57	
HER2	HER2	+	-	**4**	**4.76**	
Luminal A	Luminal A	-	-	1	1.19	
Luminal A	Luminal A	-	+	**1**	**1.19**	
Luminal A	Luminal A	+	-	**7**	**8.33**	
Luminal A	Luminal A	+	+	13	15.48	
Luminal B/HER2	Luminal B/HER2	-	-	1	1.19	**77.4**
Luminal B/HER2	Luminal B/HER2	+	-	**1**	**1.19**	
Luminal B/HER2/Ki67	Luminal B/HER2	-	-	1	1.19	
Luminal B/HER2/Ki67	Luminal B/HER2/Ki67	-	-	2	2.38	
Luminal B/HER2/Ki67	Luminal B/HER2/Ki67	+	+	1	1.19	
Luminal B/HER2/Ki67	Luminal B/Ki67	+	+	1	1.19	
Luminal B/Ki67	Luminal B/Ki67	-	-	1	1.19	
Luminal B/Ki67	Luminal B/Ki67	-	+	1	1.19	
Luminal B/Ki67	Luminal B/Ki67	+	-	**7**	**8.33**	
Luminal B/Ki67	Luminal B/Ki67	+	+	16	19.05	
Basal-like	Unclassified	-	-	1	1.19	
HER2	Luminal B/HER2/Ki67	-	-	1	1.19	
Luminal A	Unclassified	+	+	1	1.19	
Luminal A	Luminal B/HER2	+	+	1	1.19	
Luminal A	Luminal B/Ki67	+	+	2	2.38	
Luminal B/HER2	Luminal A	+	+	1	1.19	**22.6**
Luminal B/HER2/Ki67	HER2	-	-	1	1.19	
Luminal B/HER2/Ki67	Luminal A	+	+	1	1.19	
Luminal B/Ki67	Basal-like	-	-	1	1.19	
Luminal B/Ki67	Luminal A	-	-	5	5.95	
Luminal B/Ki67	Luminal A	+	+	4	4.76	

Note: Molecular subtypes shifted cases are selected with **Bold**. BCl2 transitions (No and %) are primed with gray color**. Tm** – primary tumor, **Mt** – metastases.

was associated with a better differentiation of the cancers and particularly G1 – 100% of BCL2-positive tumors, G2 – 81%, G3 – 60% [21]. This assumption is in line with our results where majority (70.2%) of BCL2 positive cases was evaluated with G2 and G3 grades. Contradictory, Binder et al. presented a significant inverse correlation between histological grading and immunoreactivity for BCL2, confirmed by Ermiah et al. too [22, 23]. In our assays a statistically significant correlation between histological grade and BCL2 was not found.

Ermiah et al. consider that patients with positive expression of BCL2 had lower recurrence rate than BCL2-negative patients and better survival after median follow-up of 47 months [23]. Recently, Yang et al. found significant relation between BCL2 negativity and good prognosis [5]. All related uniformities could have several explanations: unknown, other than anti-apoptotic function of BCL2, lack of homogeneity in studied group, differences in immunohistochemical assays and the cut-off used to define BCL2 positivity [24]. Lee et al. considered BCL2 positive case as ">0%" of stained cells and Dumontet et al. used a threshold of ">70%" positive cells [25, 26].

BCL2 is considered as modulator of hormonal/anti-

hormonal responsiveness exhibited by tumors. Binder et al. supposed that loss of BCL2 expression seems to induce the loss of hormonal regulation, increased dedifferentiation and deregulated proliferation [22]. Gee *et al.* determined that immunostaining for hormone receptors was strongly associated with that for the BCL2 protein, results which were confirmed by other researches later too [4, 27]. Authors supposed that this protein, like progesterone receptor, is under estrogen regulation via estrogen receptor. These results were confirmed also by a highly significant relationship between response to endocrine therapy and the presence of BCL2 protein. Indeed, BCL2 was more accurate predictor of response than estrogen receptor status. Patients who had a combination of BCL2 high score and elevated ER level received the greatest benefit from endocrine therapy. Our results concerning BCL2 and hormone receptors relationship are similar to Gee et al. [4].

In the literature the BCL2 was inversely related to c-erbB-2 oncoprotein (as well to epidermal growth factor receptor or EGFR) [4, 22]. Petry et al. support the concept that ERBB2 influences the expression of BCL2 family members to induce an anti-apoptotic phenotype [28]. Authors indicate that ERBB2 alters the expression of BCL2 in a way that leads to adverse prognosis. In our assays BCL2 correlated negatively with HER2 expression.

The anti-apoptotic function of BCL2 should predispose tumor to high proliferation. No associations were observed with Ki-67 proliferative status by some researches [4]. More, high proliferative activity assessed by Ki-67 correlated inversely with BCL2 expression in primary tumor in Binde et al. and Zaha et al. experiments [22, 29]. Our results are complementary to above mentioned.

CK5/6-positive breast carcinomas have a low BCL2 expression and high proliferation rate [30]. Same data arise from our study too.

Korsching et al. consider that different cellular subgroups in the female breast give rise to subgroups of breast carcinomas with different protein expression and cytogenetic alteration patterns that may be related to clinical behavior [30]. Approximately 80% of patients present hormone positive tumors. Dawson et al. established that prognostic value of BCL2 was present across molecular subtypes (ER+/Luminal, HER2+, HER2– and triple negative), and was independent of tumor size, grade and stage [31]. Cases with $ER^+/BCL2^-$ pattern had a worse prognosis than those with $ER^-/BCL2^+$.

There are several evidences which affirm the instability of molecular subtypes during metastatic process [12, 13, 32]. In our study BCL2 expression was stable throughout this transition and unexpectedly changed in the group where molecular subtype was the same at both sites. Our results are in line with Subhawong *et al.*, which reported a downregulation of BCL2 expression in metastases in Luminal A subtype [33]. But in this report, patients (17 cases) underwent many cycles of adjuvant therapy prior to study and developed resistance to hormonal therapy. We couldn't find other results concerning relationship of BCL2 expression and molecular subtypes throughout progression.

Regarding the involvement of BCL2 in metastatic process our results are in contradiction with data of other groups, which reported an increase of BCL2 expression in axillary metastases [34, 35]. Another study reported a similar BCL2 expression between the primary tumor and lymph node metastases [36]. The differences with our study are related to a number of cases (Mimori et al. with 6 cases, Arun et al. and Kristek et al. with 60 cases each), method of markers assessment (cDNA microarray at Mimori et al.) and the most important, that all of them reported BCL2 expression from classical, histopathological position of breast cancer interpretation (34-36). A direct and comprehensive comparison from molecular position of BCL2 expression between primary breast carcinomas and paired distant metastases has not been performed yet.

In summary, molecular subtypes and BCL2 expression are not stable during tumor progression and metastatic development. In the present study we established immunohistochemically that BCL2 is not influenced by subtypes' transitions. BCL2 switches were encountered only in cases with a stable HER2, Luminal A or B phenotypes. Due to its dual function, promoting cell cycle arrest and preventing apoptosis, many things concerning BCL2 implication in breast cancer progression and drug resistance remain still unclear. Its specific localization at the crossroads of several complex physiological pathways, may lead to unexpected consequences, interesting for researches, dangerous for patients.

Conflicts of interest

The authors indicated no potential conflicts of interest.

Acknowledgments

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Analysis of the legislation of the Republic of Moldova in terms of pharmaceutical security

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Abstract

Background: The pharmaceutical security can only be ensured taking into account its multidimensional and systemic character; the partial or one-sided approach will not ensure the feasibility and the sustainability of such security. A major condition to ensure pharmaceutical safety is the existence of a legislative base. The purpose of the paper is to highlight regulatory gaps affecting the pharmaceutical security of the Republic of Moldova.

Material and methods: Based on a logical analysis, all conditions, the compliance with which would ensure the pharmaceutical security of the state and of each citizen were determined. The existence or absence of legal provisions legalizing the application of various ways, methods, processes, procedures, regulations and other measures that would impose compliance with pharmaceutical security conditions was highlighted. The level of legislative coverage of the requirements for the provision of pharmaceutical security was determined. The quantification scale regarding the level of legislative coverage for the provision of pharmaceutical security was developed.

Results: The research resulted in the development of the methodical toolbox by means of which the legislative coverage level of the pharmaceutical security was identified. The calculations demonstrated that the legislation of the Republic of Moldova does not sufficiently cover the pharmaceutical security. This fact refers mostly to the insufficient regulation of the good quality of pharmaceutical services, of the proper functioning of the entire pharmaceutical system and of ensuring physical and economic availability of drugs.

Conclusions: The impact of the governmental factor in the Republic of Moldova concerning certain aspects of the pharmaceutical security was highlighted. The toolbox of methods for the analysis of the level of legislative coverage of pharmaceutical security was drawn up.

Key words: pharmaceutical system, legislation, security.

Introduction

The notion of pharmaceutical security was addressed for the first time, in the Republic of Moldova, in 2009, in a report by the Ministry of Health [6], but it should be mentioned that, in that case, the Ministry only related the results of activities to:

- Ensuring the access of the population to drugs, pointing out certain procedures (price analysis, drugs compensation, centralized procurement);
- Promotion of reasonable use of drugs;
- Development and promotion of the local drug manufacturer.

The report presented by the Ministry of Health lacked a systemic or, at least, many-sided approach to the pharmaceutical security notion. Consequently, the reports of the Ministry of Health did not include the chapter on "pharmaceutical security".

The issues related to ensuring the pharmaceutical security started being addressed nationwide from the year of 2015 [1].

The Government of the Republic of Moldova regularly started addressing various aspects of the pharmaceutical security [2, 4, 5].

At the same time, it is worthwhile mentioning that government talks are limited to certain aspects of pharmaceutical safety, without taking into account the systemic nature of this area of security [1]. To improve the situation, it is necessary to analyze the legal basis relating to pharmaceutical security of the state and of each citizen. The results of such analysis would create grounds for notifying the state authorities on the need to strengthen, in all of its aspects, and not partially, the pharmaceutical security.

The specialized literature did not offer works on the analysis of the state pharmaceutical security legislation, this fact, in tandem with the aforementioned circumstances, features the topicality of the issue being addressed.

The objective of this article is to highlight the legislative gaps affecting the pharmaceutical security of the Republic of Moldova.

Material and methods

In order to achieve the determined objective, all the legislative and normative acts, directly or indirectly regulating the pharmaceutical security, have been initially selected.

The systematization of legal and normative acts has been performed based on the following two principles:

I – law/legislation: pharmaceutical, medical, and other fields;

II – subsystems of the pharmaceutical safety system [1]: a) effectiveness, harmlessness and quality of drugs, b) availability of drugs, c) quality of pharmaceutical services and the smooth operation of the pharmaceutical system.

The list of all legislative and normative acts defining rules aimed at ensuring the pharmaceutical security includes 112 documents, among which: pharmaceutical – 78, medical – 26, other areas – 8.

Development of the analytical method

As a methodical tool for highlighting the level of legislative and normative coverage of the pharmaceutical security, the following algorithm was developed:

Step I

Determining, based on a logical analysis, all conditions, the compliance with which would ensure the pharmaceutical security of the state and of each citizen. This analysis should take into account the de facto situation of the pharmaceutical system.

Pursuant to the content analysis of the legislation and of the sub-legislative acts, as well as taking into consideration the national and international practice, the advanced experience in the field of pharmaceutical security, the conditions for ensuring this security were systematized and grouped according to the 2nd systematization principle (tab.1).

The total number of conditions necessary to ensure the pharmaceutical security shall be marked by (Cn). The number of necessary conditions can be determined as the total conditions (Cn) or the aggregate of conditions to ensure pharmaceutical security directions:

Cqd – conditions for ensuring the quality, the effectiveness and the harmlessness of drugs;
Cad – conditions for ensuring the (physical and economic) availability of drugs;
Cqa – conditions for ensuring the quality of the pharmaceutical act.

Step II

Highlighting of the existence / absence of legal provisions legalizing the application of various ways, methods, processes, procedures, regulations and other measures that would impose compliance with conditions necessary for pharmaceutical security.

Highlighting of the existence / absence of legal provisions aimed at ensuring the pharmaceutical security was performed by applying the expertise method combined with the Delphi method [3].

For this purpose a panel of experts was created randomly, representing:

– the Parliament of the Republic of Moldova;
– the Government of the Republic of Moldova;
– the Ministry of Health;
– the Medicines and Medical Devices Agency;
– Specialized pharmacy chairs of the SUMPh ”Nicolae Testemițanu”;
– Pharmacists Association of the Republic of Moldova;
– Local drug manufacturers;
– Pharmaceutical storehouses;
– Drugstores: community (private and network) and hospital drugstores.

Therefore, the panel of experts numbered 15 members, each of which was provided with a questionnaire to assess the compliance with the conditions of ensuring the legislative coverage for pharmaceutical security (tab. 1) [1], as well as the method of assessing the legislative coverage:

– Existence of the necessary legal provision – 1 point;
– Absence of the legal provision – 0 points;
– Existence of the necessary legal provision, but failure to comply with – 0.5 points;
– Inefficiency of the regulation for providing the pharmaceutical security – 0.5 points.

The legislative coverage of conditions for providing the pharmaceutical security shall be marked as:

(Pe) – existing legal provisions aimed at ensuring the compliance with the conditions necessary, in their turn, to provide the pharmaceutical security. Indicator (Pe), as well as (Cqa) may be highlighted for the total number of existing rules or for the directions of pharmaceutical security provision:

Peqd – effective legal provisions covering the conditions of pharmaceutical security provision by ensuring the quality, the efficiency and the harmlessness of drugs.

Pead – effective legal provisions ensuring the physical and economic availability of drugs;

Peqa – effective legal provisions ensuring the quality of the pharmaceutical act.

Step III

Determining the degree of legislative coverage of requirements for pharmaceutical safety provision. This indicator shall be marked as (LCp) – for the total number of existing legal provisions and necessary conditions, and for the three directions for providing pharmaceutical security, accordingly:

LCqd = Peqd / Cqd (1) – indicator of legislative coverage of pharmaceutical security provision by ensuring the quality, efficiency and harmlessness of drugs;

LCad = Pead / Cad (2) – indicator of legislative coverage of pharmaceutical security provision by ensuring the physical and economic availability of drugs;

LCqa = Peqa / Cqa (3) – indicator of legislative coverage of pharmaceutical security provision by ensuring the quality of the pharmaceutical act.

Step IV

Development of the quantification scale for the degree of legislative coverage for the pharmaceutical security provision. Taking into consideration that the value of indicator (ALn) may vary between ”zero” and ”1”, we undertook to develop a clear assessment scale.

For a clearer perception of the indicator ALn, it was proposed to multiply its value by 10, thus, the indicator amplitude will fall in the range of 1-10. For the quantification, the following appreciation scale was suggested:

LCp = 0 - null;
LCp = > 0 … 2 – vulnerable;
LCp = > 2 ... 5 – insufficient;
LCp = > 5 ... 8 – moderate;
LCp = > 8 …< 10 – good;
LCp = 10 – total.

Therefore, the final formula for computing the degree

of legislative coverage for the state pharmaceutical security provision (LCp) is as follows:

$$LCp = \frac{P_e}{C_n} \times 10 \quad (4),$$

in which:

Pe – the number of existing legal provisions aimed at pharmaceutical security provision;

Cn – the number of necessary conditions aimed at pharmaceutical security provision.

Results

Experts expressed their opinions, assessing the existence / absence of the respective legal provision and / or the existence and failure or inefficiency of the existing provision. The opinions of all the experts coincided for 39 conditions (90.7%). The opinions of three experts (20%) were different for four legislative conditions of pharmaceutical security provision (1.5, 2.12, 3.8 and 3.9). Following the application of the Delphi method, a consensus was reached and the as-

Table 1

Conditions for pharmaceutical security provision

No	Conditions for pharmaceutical security provision	Legislative coverage
1	2	3
I.	**Ensuring efficiency, harmlessness and good quality of drugs**	
1.1.	Conducting drug development research on ethical principles aimed at achieving health benefits;	0,5
1.2.	Consistent and efficient operation of the authorisation procedure for drugs manufacturing in domestic enterprises;	1
1.3.	Compliance with Good Practice Rules: GLP (good laboratory practice), GCP (good clinical practice), GMP (good manufacturing practice) for drugs;	0,5
1.4.	Ensuring quality of drug substances and excipients used in the manufacture and preparation of drugs;	1
1.5.	Consistent and efficient operation of the service of pharmacovigilance;	1
1.6.	Ensuring transparency in the R&D process in the development of new drugs;	0
1.7.	Ensuring the efficiency of the licensing process (expertise, approval, registration) of drugs marketing;	1
1.8.	Ensuring the consistency and efficiency of the process of licensing drug imports;	1
1.9.	Prevention of placing drugs not subject to quality control on the pharmaceutical market	1
1.10.	Availability and continued application of measures to prevent the placement on the pharmaceutical market of counterfeit drugs	0
1.11.	Ensuring consistent storage (GDP, GSP) and consistent transportation (GTP) of drugs.	0
II.	**Ensuring physical and economic availability of drugs**	
2.1.	Uniform location of community drugstores throughout the country according to demographic and geographic rules;	0
2.2.	Ensuring the presence of essential drugs, including compensated drugs in/at: • State drug nomenclature; • National Catalogue of manufacturer prices; • Drug storehouses; • Community drugstores; • Healthcare institutions (according to the Institutional Pharmaco-therapeutical Form and national clinical protocols).	0,5
2.3.	Establishing accountability for the availability /lack of essential drugs in the pharmaceutical market;	0
2.4.	Development and implementation of the orphan drugs concept;	0
2.5.	Providing assistance with medications for people during the day and 24 hour-emergency assistance;	0
2.6.	Providing full information regarding the availability /lack of drugs at community drugstores /drug storehouses;	0,5
2.7.	Existence of the rule regarding the minimum required range of drugs within community drugstores and drug storehouses;	0,5
2.8.	Establishing a legal provision prohibiting unjustified refusal of drug delivery from the pharmaceutical storehouse to drugstores and medical institutions;	0

2.9.	Existence of an efficient mechanism of drug pricing;	0,5
2.10.	Providing professional negotiation of manufacturer prices;	0,5
2.11.	Ensuring effective functioning of the mechanism of drug public procurement for the needs of public medical institutions;	0,5
2.12.	Ensuring total transparency and excluding the conflict of interest from the process of public procurement of drugs;	0
2.13.	Continuous extension of the list of compensated drugs;	0,5
2.14.	Creation of incentive mechanisms for ensuring the availability of generic drugs on the pharmaceutical market.	0
III	**Ensuring good quality of pharmaceutical services and of good operation of the pharmaceutical system**	
3.1.	Ensuring compliance with essential pharmaceutical services (consistency with the accreditation standards and standard operating procedures)	0,5
3.2.	Combating unfair competition in the pharmaceutical market, banning monopoly	
3.3.	Establishing enhancers for the implementation of advanced pharmaceutical services	0
3.4.	Providing uninterrupted/timely information on the pharmaceutical act	0,5
3.5.	Ensuring the compliance with the requirements related to the professional level of specialists engaged in pharmaceutical activity	0
3.6.	Compliance with standards of good pharmacy practice (GPP) and standards of good distribution practice (GDP)	0,5
3.7.	Strengthening the functionality and efficiency of the Pharmaceutical Inspectorate	0,5
3.8.	Regulating the principles of reasonable use of drugs	0
3.9.	Streamlining the concept and the procedures for the accreditation of pharmaceutical enterprises;	0
3.10.	Amending the procedure for authorising (licensing) the pharmaceutical activity and its professional strengthening;	0
3.11.	Ensuring the compliance with the rules for the ethical promoting of drugs;	0
3.12.	Ensuring the prevention of conflicts of interest and of corruptibility in all processes, procedures and functions comprised in the notion of pharmaceutical activity;	0
3.13.	Strengthening the procedures for the safe disposal of expired, refuse or deteriorated drugs;	1
3.14.	Due exercise of the professions of pharmacist and assistant pharmacist;	0,5
3.15.	Strengthening the role of professional organisations of pharmacists in order to ensure the high quality of pharmaceutical services and the smooth functioning of the entire pharmaceutical system;	0
3.16.	Establishing and promoting the principles for the collaboration between doctors and pharmacists to the benefit of the patient;	0
3.17.	Effective contributions to the prevention and combating of drug abuse and drug dealing;	0,5
3.18.	Interaction regarding the development of all the dimensions of the clinical pharmacy concept.	0

sessment results coincided for the whole panel of experts (tab. 1, section 3).

The data provided at point 3, table 1 show the degree of legislative coverage of the conditions necessary for ensuring pharmaceutical security in the Republic of Moldova (tab. 2).

The data in table 2 indicate that the legislative coverage of the pharmaceutical security of the Republic of Moldova is insufficient. All the sectors need to be completed and require new legal provisions, but the most poorly regulated is the quality of pharmaceutical services and the functioning of the pharmaceutical system as well as the ensuring the availability of drugs. The results obtained point to legislative gaps that affect the pharmaceutical security and may serve as benchmarks in the legislative activity regarding the pharmaceutical field.

Table 2

Level of legislative coverage of the pharmaceutical security of the Republic of Moldova

Sectors	Legislative coverage				Level of coverage
	Existence of rules	Lack of rules	"Non-compliance", "insufficiency"	Points accumulated	
1. Ensuring efficiency, harmlessness and high quality of drugs	6	3	2	7	6,63 moderate
2. Ensuring physical and economical availability of drugs	-	7	7	3,5	2,50 insufficient
3. Ensuring the high quality of pharmaceutical services and the smooth functioning of the pharmaceutical system	1	10	6	4	2,22 insufficient
The entire system	7	20	15	14,5	3,37 insufficient

Conclusions

1. The impact of the governmental factor in the Republic of Moldova concerning certain aspects of the pharmaceutical security was highlighted;

2. The toolbox of methods for the analysis of the level of legislative coverage of pharmaceutical security was drawn up;

3. The insufficient legislative coverage of pharmaceutical security was proven and the legislative gaps related to pharmaceutical security and, namely, the conditions necessary for its ensuring were pointed out: lacking – 20 rules; inefficient or not complied with – 15 rules.

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Homocysteine and recurrent miscarriage

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Abstract

Background: It is known that etiological structure of recurrent miscarriage has genetic, anatomical, infectious and immunological factors; however, the cause of recurrent miscarriage in 50-60% of cases is not completely clear. Homocysteine is a sulfur-containing intermediate product in the normal metabolism of methionine. Development mechanisms of vascular complications of hyperhomocysteinemia are currently being intensively studied. Hyperhomocysteinemia affects a number of mechanisms involved in thrombogenesis including coagulation cascade, vessel-thrombocytic section, oxidation-reduction reactions, endothelium, and vascular smooth muscle cells and is associated with an increased risk of adverse outcomes in pregnancy.
Materials and methods: The study included 50 women who had experienced the loss of at least two consecutive pregnancies. The level of the total serum homocysteine was measured via the chemiluminescent method.
Results: We found that plasma homocysteine concentration < 10 μmol/l was found in 16 patients (32,0%, 95% CI 19,07 – 44,93), 9 patients (18.0%, 95% CI 7,36 – 28,64) had a fasting plasma homocysteine between 10 μmol/l to 12 μmol/l and 25 patients (50.0%, 95%CI 36,15 – 63,85) had significantly high total serum homocysteine values. Among them, 23 patients (46.0%, 95%CI 32,19 – 59,81) had the concentration between 12 – 30 μmol/l and 2 patients (4,0%, 95%CI -1,43 – 9,43) had the concentration > 30μmol/l. The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception. In the current study, 40 women (80.0%, 95% CI 68,92 – 91,08) have become pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.
Conclusions: The prevalence of hyperhomocysteinemia was more in unexplained primary early recurrent miscarriages. The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception and 40 women (80.0%, 95% CI 68,92 – 91,08) became pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.

Key words: homocysteine, spontaneous abortion, recurrent abortion, vitamin B supplementation.

Introduction

Spontaneous abortions (SA) are one of the most frequent pregnancy disorders and are defined as the loss of fetal product before 22 weeks of gestation [2]. SA is observed in approximately 15-20% of total pregnancies [3], although this proportion could even be higher. Roughly 1 in 5 pregnancies would, thus, end in spontaneous abortion, the majority until the gestational age of 12-14 weeks, and 1-2% would account for recurrent spontaneous abortions [2]. Recurrent miscarriage (RM) is defined as the occurrence of three or more consecutive losses of pregnancy [5]. However, many clinicians define RM as two or more losses and this increases the percentage of RM from 1% to 5% of all couples trying to conceive. RM is a very frustrating condition for both the couple and the clinician, because it is difficult to find a distinct reason for the repeated failure to sustain a pregnancy and eventually have a successful pregnancy outcome. The causes involved in RM are numerous: anatomical, endocrine, immunological, infectious, environmental, and genetic or combinations of these [3]. Not infrequently, despite all medical advances, these causes cannot be identified with certainty, and the abortion declared idiopathic remains the final diagnosis in approximately 50% of cases [2].

During the past decade, the list of candidate causes of recurrent pregnancy loss (RPL) has grown rapidly. During the early 1990s, an elevated plasma homocysteine concentration, which has been described as a risk factor for arteriosclerosis, venous thrombosis, neural tube defects, placental abruption or infarction and preeclampsia, was also suggested to be associated with RPL [8,10].

Several reports have clearly shown an association of elevated homocysteine concentrations and obstetric diseases that are connected with vascular disorders of pregnancy or of the utero-placental unit [3].

Nelen et al. [8] studied women with repeated miscarriages and found a direct relationship between high levels of homocysteine and defective chorionic villous vascularization: early miscarriages might be explained by the damage that excess homocysteine may cause on chorionic and decidual vessels leading to defective implantation of the embryo. Placental development in early pregnancy may be negatively influenced by increased maternal homocysteine concentrations [1]. Experimental studies revealed that moderately elevated homocysteine concentrations may induce cytotoxic and oxidative stress, leading to endothelial cell impairment [1, 6, 13, 16]. Additionally, exposure of trophoblast cells to homocysteine may increase cellular apoptosis and lead to inhibition of trophoblastic function [1,3]. Recently, Di Simone et al. [3] provided the first demonstration that human placenta is a target for homocysteine and suggested that trophoblast death might represent one mechanism by which homocysteine causes pregnancy complications related to placental diseases.

The aim of this study was to evaluate the level of plasma homocysteine concentration in a group of women with recurrent pregnancy loss and to investigate the association between them in order to look for hypothetical possibilities of therapeutic interventions.

Material and methods

The study included 50 women of fertile age with at least two consecutive miscarriages in the first and second trimester of gestation. All the enrolled cases have no identified causes of abortions. The patients were recruited at the Department of Obstetrics and Gynecology No 2, Nicolae Testemitsanu State University of Medicine and Pharmacy and at the medical center "Repromed"of Chisinau city, the Republic of Moldova from April to September in 2014 to 2016. The cases were identified and selected when they visited the above-mentioned hospitals for investigation of two or more consecutive unexplained terminations of pregnancy. Women with clinical diagnosis of metabolic deseases, renal insufficiency and a woman who took vitamin supplements were excluded from this study. Socioeconomic data (schooling and occupation) and obstetrics data were assessed by questionnaire. Written informed consent was obtained from all women before participation. The study was approved by the Ethics Committee of the Nicolae Testemitsanu State University of Medicine and Pharmacy, Chisinau, the Republic of Moldova.

The measurements of plasma homocysteine concentration were performed using chemiluminescence method. Blood samples were drawn from women using sterile tubes with anticoagulant (EDTA) and the plasma for the analysis of total homocysteine concentration was centrifuged immediately.

The lowering homocysteine level therapy included vitamin supplementation (folic acid, vitamin B12 and vitamin B6) and was also recommended to eat a diet rich in vitamins, as a part of preconception care.

The Microsoft Excel application (Microsoft Office 2010) and online OpenEpi application, version 2.3.1 [9] were used for the statistical analysis. Parametric variables were compared by Student's *t*-test and the 95% confidence interval (CI) was calculated.

Results and discussion

The median age of the 50 women in the study group was 29,52±5.89 years, ranging from 20 to 42 years old. The documentation of patients' places of residence identified that 27 respondents (54,0%, 95% CI 40,19 – 67,81) were from urban area and 23 respondents (46,0%, 95% CI 32,19 – 59,81) were from rural area. The study has included 4 students (8,0%, 95% CI 0,49 – 15,51), 28 respondents (56,0%, 95% CI 42,25 – 69,75) were engaged in an intellectual type of work, 13 respondents (26,0%, 95% CI 13,49 – 38,51) were workers and 5 respondents (10,0%, 95% CI 1,69 – 18,31) performed housework.

The gestational age, when the abortions occurred, was divided into the first and the second trimester of gestation and mixed when there were intersessions between them. Our data show that in 44 women (88,0%, 95% CI 79,0 – 97,0), the abortion occurred in the first trimester, in 1 woman (2,0%, 95% CI -1,43 – 9,43) 22 (25%) the abortion occurred in the second trimester and 5women (10,0%, 95% CI 1,69 – 18,31) were included in the mixed abortion group, because they presented these events in the first and second trimester during their pregnancies (fig. 1). We can conclude that 49 patients (98,0%, 95% CI 94,12 – 101,88) had early RPL and 6 patents (12,0%, 95% CI 3,0 – 21,0) had late RPL.

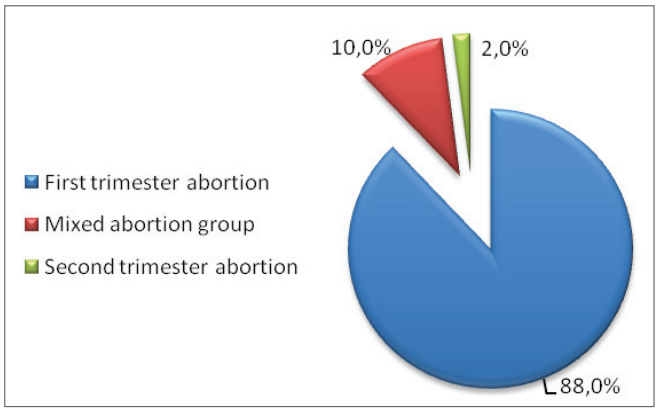

Fig. 1. The gestational – age distribution of spontaneous abortion in the study (%).

The median abortion number in the study group was 2,32±0,62, ranging from 2 to 5 abortions, in which 37 women (74,0%, 95% CI 61,85 – 86,15) had two spontaneous abortions; 11 (22,0%, 95% CI 10,52 – 33,48) of them had three spontaneous abortions, 1 (2,0%, 95% CI -1,43 – 9,43) of them had four spontaneous abortions and, also, 1 (2,0%, 95% CI -1,43 – 9,43) of them had five spontaneous abortions (fig. 2).

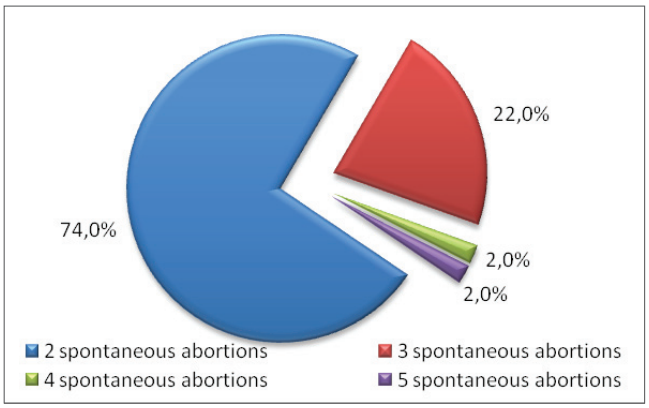

Fig. 2. The frequency of spontaneous abortions in the study (%).

Also, 33 patients (66,0%, 95% CI 52,87 – 79,13) were classified as suffering from primary RPL when they had never had a live birth before and 17 patients (34,0%, 95% CI 20,87-47,13) suffering from secondary RPL when they had recurrent losses following a successful pregnancy (fig. 3).

The median fasting total plasma homocysteine concentration was 13,36±1,0 μmol/l, ranging from 2,19 mmol/l to

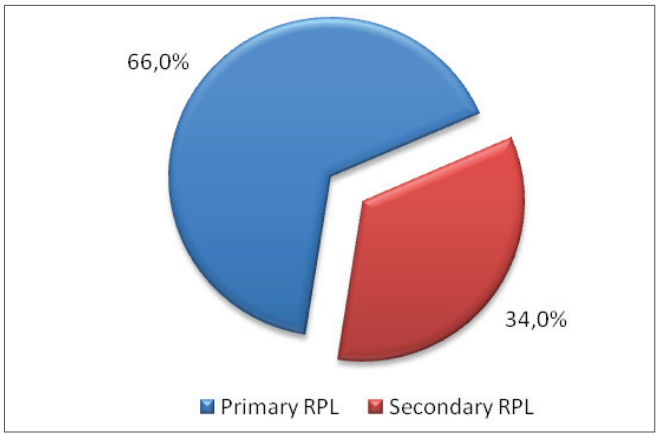

Fig. 3. Type of pregnancy loss in recurrent miscarriage in the study (%).

34,7 mmol/l.

According to the D.A.CH. – Liga Homocysteine (German, Austrian, and Swiss Homocysteine Society) fasting total serum homocysteine (< 10 µmol/l) is considered safe and should be the target level during homocysteine-lowering treatment [13]. Vollset et al. [16] reported that the women with the highest level of homocysteine (greater than 10 µmol/l) have an adjusted risk for preeclampsia. Ronnenberg et al. [12] demonstrated that the risk of preterm birth was nearly 4-fold higher among women with preconception homocysteine concentrations ≥ 12.4 µmol/l compared to women who had lower homocysteine concentrations. Urban et al. [15] mentioned that the mean serum homocysteine concentration was 11,50 µmol/l in the group of patients with intrauterine fetal growth restriction compared to the group of normal pregnacies who had lower homocysteine concentrations – 9,58 µmol/l.

Taking in consideration the information mentioned above, for these analyses, we defined elevated homocysteine as a plasma concentration of homocysteine > 10 µmol/l.

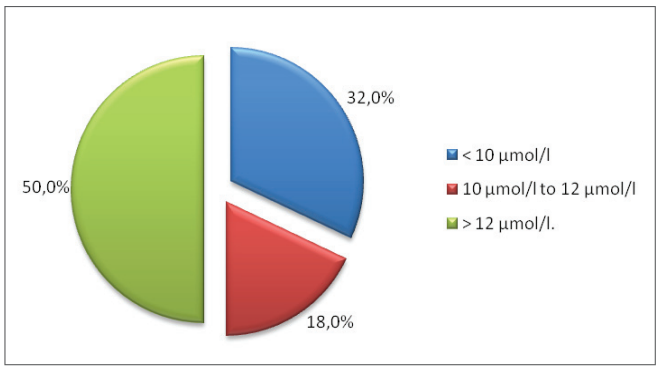

Fig. 4. Distribution of the plasma homocysteine level in the study group (%).

The results of our investigations showed that plasma homocysteine concentration < 10 µmol/l was found in 16 patients (32,0%, 95% CI 19,07 – 44,93), 9 patients (18,0%, 95% CI 7,36 – 28,64) had a fasting plasma homocysteine between 10 µmol/l to 12 µmol/l and 25 patients (50,0%, 95%CI 36,15 – 63,85) had significantly high total serum homocysteine values (fig. 4). Among them, 23 patients (46,0%, 95%CI 32,19 – 59,81) had the concentration between 12 – 30 µmol/l and 2 patients (4,0%, 95%CI -1,43 – 9,43) had the concentration > 30µmol/l.

Ueland et al. suggested that the correlation between preconceptional total serum homocysteine and total serum homocysteine during pregnancy points to the possibility that preconceptional total serum homocysteine may predict homocysteine-associated pregnancy complications [14]. As a result, we defined that 34 patients (68,0%, 95% CI 55,07 – 74,93) had elevated serum homocysteine (higher than 10 µmol/l, according to our initial consideration).

In this context, it was evaluated total plasma homocysteine concentration in relation to the type of recurrent miscarriage in women with unexplained pregnancy loss (tab. 1).

Table 1

Total plasma homocysteine concentration in relation to the type of recurrent miscarriage in the study group

Total plasma homocysteine concentration (µmol/l)	Primary RPL	Secondary RPL	t	p
	14,48±1,49	11,55±0,94	1.6631	>0.05

The results of our investigations showed that homocysteine levels in the blood serum of patients suffering from primary RPL was higher (14,48±1,49 µmol/l) compared to patients suffering from secondary RPL (11,55±0,94 µmol/l) (p >0.05).

We found that the total serum homocysteine concentration in the study group was higher (13,50±1,01 µmol/l) in the patients with a history of early RPL (tab. 2) than in the patients with a history of late RPL (9,98±1,69 µmol/l) (p >0.05).

Table 2

Total plasma homocysteine concentration according to the stage of pregnancy loss in the study group

Total plasma homocysteine concentration (µmol/l)	Early RPL	Late RPL	t	p
	13,50±1,01	9,98±1,69	1.7879	>0.05

Patients who had ≥3 abortions showed a significantly higher total serum homocysteine concentration (15,76± 1,82 µmol/l) when compared to patients who had 2 spontaneous abortions (12,51±1,18 µmol/l) (p >0.05) (tab. 3).

Table 3

Total plasma homocysteine concentration according to the number of abortions in the study group

Total plasma homocysteine concentration (µmol/l)	2 spontaneous abortions	≥3 spontaneous abortions	t	p
	12,51±1,18	15,76±1,82	1.49783	>0.05

Hyperhomocyteinemia can cause obstetrical diseases that are connected with vascular disorders of pregnancy or the uteroplacental unit [8]. Gris et al. [5] reported an association between increased levels of homocysteine and a first early pregnancy loss. Kumar et al. [6] considered elevated homocysteine levels to be a risk factor for recurrent pregnancy loss.

In the current study, we found that the incidence of hyperhomocysteinemia was higher in women with unexplained primary early recurrent miscarriages.

Nutritional factors, particularly consumption and seric concentration of folate, vitamin B_{12} and B_6, seem to be the major parameters in homocysteine metabolization. Deficiencies, isolated or combined, of vitamins involved in the various pathways of homocysteine metabolism would be important markers for hyperhomocysteine [7].

An adequate intake of at least 400 μg of folate per day is difficult to maintain even with a balanced diet, and high-risk groups often find it impossible to meet these folate requirements. The bioavailability of dietary folates is 55 %. As the recommendation to eat a healthy diet has little or limited impact on elevated homocysteine levels, (folate)-fortified foods and/or vitamin supplements are rational and therefore recommended. [13]

Maternal hyperhomocysteinemia is related to birth defects, including neural tube defects (NTDs), orofacial clefts, clubfoot, and Down syndrome. Folic acid supplements in the periconceptual period and the first few weeks of pregnancy reduce the risk of NTDs [10,11]. For this reason, all women of childbearing age should have a folate intake of at least 400 μg/day [10,17].

Vitamin supplementation continues to be a recommendable option for prophylaxis of hyperhomocysteinemia. Dosages for prophylaxis are given in Figure 5 (low-dose supplementation: folic acid – 0.2 to 0.8 mg/day; vitamin B_{12} – 3 to 100 μg/day; vitamin B_6 – 2 to 25 mg/day). If this supplementation regimen lowers plasma homocysteine to <10 μmol/l within 4 weeks, repeated measurements of plasma homocysteine should be obtained first every 6 months and later on once a year. If response (plasma homocysteine reduction) is still inadequate, the dosage of folic acid should be increased to, say, 1 to 5 mg of folic acid per day (while supplementation with vitamin B_{12} and vitamin B_6 can be continued unchanged for some time). Repeated determinations of plasma homocysteine should be performed at 4-week intervals. [13]

There are biologically plausible reasons that increased total serum homocysteine may be related to adverse pregnancy outcomes. Increased total serum homocysteine may directly or indirectly cause endothelial dysfunction, impair neurolation, reduce microfilament synthesis, inhibit DNA methylation and alter gene expression, and reduce *S*-adenosylmethionine-dependent methylation reactions [10].

Regarding plasma levels of homocysteine, subjects were divided into two groups: group I – women with plasma homocysteine concentration < 10 μmol/l (n = 16) and group II – women with plasma homocysteine concentration > 10 μmol/l (n = 32).

In our study, we recommended a complex of B vitamin supplementation (folic acid – 0,4 mg/day; vitamin B_{12} – 3 to 100 μg/day; vitamin B_6 – 2 to 25 mg/day) for women with plasma homocysteine concentration < 10 μmol/l and a complex of B vitamin supplementation (folic acid:1 – 5 mg; vitamina B_{12}: 100 – 600 μg; vitamina B_6: 6 – 25 mg) for women with plasma homocysteine concentration > 10 μmol/l. Also, everybody was recommended to eat a diet rich in vitamins.

The complex of B vitamin supplementation was recommended at least 2 to 3 months before conception. As a result, in the first group only 10 women (62,5%, 95% CI 38,78 – 86,22) from 16 women became pregnant. In the second group, 32 women (94,11%, 95% CI 48,01 – 140,21) became

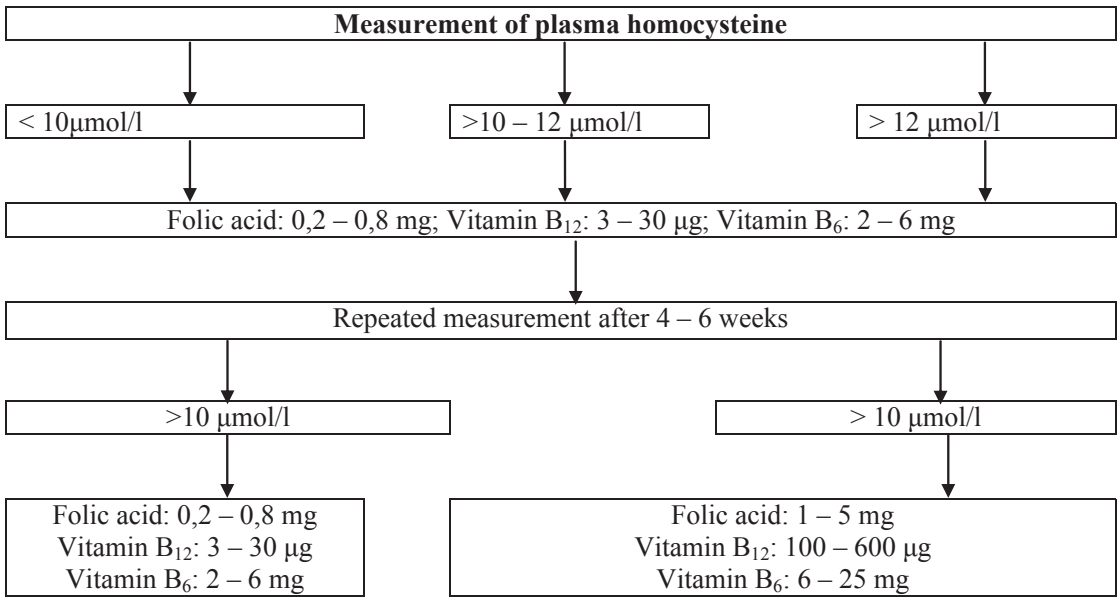

Fig. 5. Decision tree for diagnosis and prophylaxis/treatment of hyperhomocysteinemia [13].

pregnant, of which 2 women had a miscarriage in the first trimester of pregnancy. All the pregnant patients passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy. However, because of the small number of selected patients, our data should be confirmed by further studies based on larger population.

Conclusions

1. Our study provides data concerning the involvement of homocysteine in women with RPL without other causes of recurrent abortion. We found that the incidence of hyperhomocysteinemia was higher in women with unexplained primary early recurrent miscarriages.

2. The complex of B vitamin supplementation and, also, a diet rich in vitamins was recommended at least 2 to 3 months before conception.

3. In the current study, 40 women (80,0%, 95% CI 68,92 – 91,08) became pregnant, passed the critical periods for pregnancy loss and continued the folate intake during the pregnancy.

4. Because of the small number of selected patients, our data should be confirmed by further studies based on larger population.

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Metabolic syndrome as a risk factor for ischemic stroke

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Abstract

Background: Ischemic stroke is the leading cause of disability and a major cause of mortality worldwide. It is predominantly seen in the elderly and in patients with the metabolic syndrome (MS) [1, 2].

Material and methods: A "case-control" study was performed on 125 subjects with ischemic stroke and on 300 subjects without stroke. After the patients or their relatives signed an informed written consent, according to the declaration of Helsinki, the baseline data was collected by questionnaire. All subjects underwent a complete clinical examination and ultrasound examination of the extracranial carotids. Ischemic stroke diagnostic was made by a neurologist and confirmed by a brain CT scan. MS diagnostic was made according to the diagnostic criteria of the American Cardiology Association (AHA), the National Heart, Lung and Blood Institute (NHLBI) and the International Diabetes Federation (IDF) (2009).

Results: Fifty-four percent of patients and 36% of controls had metabolic syndrome criteria according to AHA, NHLBI, IDF (OR: 2.1; CI (1.1, 3.1), p=0.05). The prevalence of atherosclerotic plaques at the level of the extracranial carotid section was significantly higher in patients with stroke compared to the control group (67.2 % vs. 20,0%).

Conclusions: In our study generally metabolic syndrome was higher in stroke patients but different components of this syndrome were significantly high either. So management of individual components of the metabolic syndrome is recommended, including lifestyle measures (exercise, appropriate weight loss, proper diet) and pharmacotherapy (medications for BP lowering, lipid lowering, glycemic control, and antiplatelet therapy).

Key words: metabolic syndrome, stroke, risk factor, atherosclerosis.

Introduction

Stroke is the second major cause of death worldwide and may soon become the leading cause of death [1]. The ischemic stroke risk factors are classified as modifiable and unmodifiable ones and include arterial hypertension, diabetes mellitus, dyslipidemia, cigarette smoking, alcohol consumption, oldness, gender, etc. Furthermore, metabolic syndrome (MS) is known as an independent risk factor of vascular disease and stroke either [1, 2]. MS confers a 5-fold increase in the risk of type 2 diabetes mellitus and 2-3-fold the risk of developing cardiovascular disease over the next 5 to 10 years [3]. Further, patients with the MS are at 2- to 4-fold increased risk of stroke, a 3- to 4-fold increased risk of myocardial infarction, and 2-fold the risk of dying from such an event compared with those without the syndrome [4, 5]. Worldwide prevalence of MS ranges from <10% to as much as 84%, depending on the region, urban or rural environment, composition of the population studied, and the definition of the syndrome used [6, 7]. The IDF estimates that one-quarter of the world's adult population has the MS [8].

This study was preformed to evaluate the metabolic syndrome rate in ischemic stroke patients compared to controls.

Material and methods

A "case-control" study was performed on 125 subjects with ischemic stroke that were examined in the Cerebrovascular Diseases Neurology Department of the Emergency Medicine Institute, in the period of March 2015–July 2015. 300 subjects without stroke were examined during an epidemiological study of risk factors for stroke in the population of the Republic of Moldova from October till November 2015.

The patients were selected according to the MS diagnostic criteria of the American Cardiology Association (AHA), the National Heart, Lung and Blood Institute (NHLBI) and the International Diabetes Federation (IDF) (2009). After the patients or their relatives signed an informed written consent, according to the declaration of Helsinki, the baseline data was collected by questionnaire. All subjects underwent a complete clinical examination and ultrasound examination of the extracranial carotids. Ischemic stroke diagnostic was made by a neurologist and confirmed by a brain CT scan.

Definition of metabolic syndrome

We used revised American Cardiology Association, the National Heart, Lung and Blood Institute and the International Diabetes Federation (2009), which defined metabolic syndrome as the presence of ≥3 of the following:

- Abdominal obesity as determined by waist circumference ≥94 cm for men and ≥80 cm for women,
- Triglycerides ≥150 mg/dL (≥1,7 mmol/l),
- HDL cholesterol <40 mg/dL (<1,0 mmol/l) for men and <50 mg/dL (<1,3 mmol/l) for women,
- BP ≥130/≥85 mm Hg, Fasting glucose ≥100 mg/dL (≥5,6 mmol/l).

Statistical analysis

Data were analyzed by SPSS version 16.0; chi-square test and t-student were used for comparisons between two groups.

Results

Mean age of the studied groups was 66 years in case group and 50.25 in controls. Fifty-four percent of patients and 34% of controls had metabolic syndrome criteria according to AHA, NHLBI, IDF (OR: 2.1; CI (1.1, 3.1), p=0.05).

Totally prevalence of MS in women was more than in men in control subjects (54.4% women in patients with stroke vs. 66,0% women in control group) but there was no significant difference between them.

Fifty-seven percent of patients and 30% of controls had basal plasma glucose level higher than 5,6 mmol/l (p-value =0.001) (tab. 1).

Table 1

Characteristics of patients with ischemic stroke and control group

	Patients (125 subjects)	Controls (300 subjects)	P value
Mean age	66,00 years	50,25 years	0,001
Female	68 (54,4%)	198 (66%)	
Male	57 (45,6%)	102 (34%)	
Percent of positive metabolic syndrome	68 (54,4%)	108 (36%)	0,001
Percent of high blood pressure	113 (90%)	132 (44%)	0,001
Percent of low HDL-cholesterol	50 (40%)	31 (10%)	0,001
Percent of high waist circumference	102 (82%)	165 (55%)	0,001
Percent of high tryglicerides	35 (28%)	68 (23%)	0,05
Percent of high basal plasma glucose level	71 (57%)	92 (30%)	0,001

The tryglicerides level was significantly higher in patients than in controls (p-value =0.05). The HDL level in patients was significantly lower than controls (40% vs. 10%) (p-value =0.001).

Waist circumferences quantified in patients were higher than controls, 82% of patients and 55% of controls had high waist circumference (p-value= 0.001).

Ninety percent of patients and 44% of controls had hypertension (P-value =0.001).

Atherosclerotic plaques at the level of the extracranial carotid section were found in 67.2% (84 subjects) of the participants from the basic group compared to 20.0% (60 subjects) from the control group.

Discussion

The result of our study showed significant differences in metabolic syndrome among stroke patients and control subjects.

Although it is not clear how MS can increase the rate of vascular diseases and its mechanism is in doubt but MS is known as a vascular disease independent risk factor and associates with high risk of vascular events in ischemic stroke patients in many studies.

In our study generally metabolic syndrome was higher in stroke patients but different components of this syndrome were significantly high either.

Prevalence of hypertension was significantly high in stroke patients compared to controls (90% vs. 44%) and basal plasma glucose level was more (>5,6 mmol/l) in stroke patients (57%) than in controls (30%).

In this study, it was demonstrated that the frequency of MS in women was not more than in men, like other studies the correlation of vascular disease and MS proved to be in both genders. Moreover, male patients with the metabolic syndrome had higher mortality than others [8].

Our finding consists of other studies that showed more important roles of metabolic syndrome and higher risk of stroke in MS patients in both genders compared to controls [8] according to these studies, the frequency of stroke became two times higher.

As MS increases intra- and extra-cranial atherosclerosis it can be associated with higher risk of stroke, a lot of studies showed that half of patients with symptomatic intra- and extra- cranial atherosclerotic disease had metabolic syndrome [9, 10, 11, 12].

The metabolic syndrome is currently more frequent and a large number of people worldwide are in danger [3, 7, 8]; therefore, it is necessary to pay attention to the frequency of this syndrome in order to control the vascular disease prevalence, especially in the elderly.

Conclusions

In MS the risk of cerebrovascular diseases is multifactorial and its early detection and its treatment can prevent vascular events. As the frequency of metabolic syndrome in stroke patients is higher than in controls, it is important to manage the individual components of the metabolic syndrome, including lifestyle measures (exercise, appropriate weight loss, proper diet) and pharmacotherapy (medications for blood pressure lowering, lipid lowering, glycemic control, and antiplatelet therapy).

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Markers of apoptosis and oxidative stress in congestive heart failure

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Abstract

Background: The aim of this work is to study the markers of apoptosis (granzyme B) and oxidative stress (nitric oxide), as well as indicators of endothelial damage - cystatin C and lipid metabolism in patients with congestive heart failure, and to identify the relationship between these parameters.

Material and methods: The study included 114 patients (men and women) with congestive heart failure (CHF), out of which 41 patients with CHF, 39 patients with CHF and diabetes mellitus type 2, and 34 patients with CHF with metabolic syndrome. Biochemical parameters were measured with the help of reagent sets produced by "Human" company (Germany), the contents of granzyme B and cystatin C – with the help of commercial sets produced by "USCN Life Science Inc" company (China), while nitric oxide concentration was determined using a commercial kit by "R&D System"

Results: Analysis of indicators of apoptosis factors and oxidative stress in studied patients revealed a substantial increase in patients with heart failure in the presence of diabetes mellitus type 2, compared to two other groups of patients. The concentration of cystatin 3 in patients with diabetes mellitus type 2 increased significantly with *p <0,05.

Conclusions: The studied parameters allow us to suggest that complications in CHF are due to the intensity of oxidative stress, apoptosis and atherogenesis, and are interconnected with biochemical changes in lipid and carbohydrate metabolism.

Key words: apoptosis, metabolic syndrome, congestive heart failure, diabetes mellitus.

Introduction

Worldwide the prevalence and characteristics of the metabolic syndrome (MS) are linked to immobile lifestyle, consumption of high-calorie "fast food" products, as well as global unfavorable environment. According to recent research conducted by ARIC (Atherosclerosis Risk in Communities Study), the prevalence of metabolic syndrome among men and women constitutes 23% and 24%, respectively [1]. In the age group from 25 to 45, this pathological state makes up 35-53,1%. In patients with pathological syndrome the risk of cardiovascular complications increases, including myocardial infarction and stroke, which results in mortality, in 80% of cases MS leads to the development of diabetes mellitus [2].

Currently an increase in prevalence of diabetes mellitus type 2 (DM-2) is observed in economically developed countries. It is known that in patients with DM-2 the risk of development of vascular pathology, including coronary artery disease, increases by 2-4 times. In turn, combination of DM-2 and heart failure boosts the risk of fatal outcome by 4 times. In MS, as well as in DM, the risk of development of cardiovascular complications, including myocardial infarction and stroke, is very high [3].

Apoptosis plays an important role in the pathogenesis of cardiovascular diseases, such as atherosclerosis, cardiac ischemia and congestive heart failure [4]. Thus, the extent of damage to cardiomyocytes is determined by such pathological processes as apoptosis, oxidative stress, as well as metabolic changes. The aim of this work was to study the markers of apoptosis (granzyme B) and oxidative stress (nitric oxide), as well as indicators of endothelial damage – cystatin C and lipid metabolism in patients with CHF and to identify the relationship between these parameters.

Material and methods

The research included 114 patients (men and women) with congestive heart failure (CHF). Patients were divided into 3 groups: I group – 41 patients with CHF, II group – 39 patients with CHF and DM-2, and III group – 34 patients with CHF with metabolic syndrome. 10 healthy patients comprised the control group. During the stay in hospital laboratory and functional studies were conducted in all patients, antianginal and antiplatelet therapy was appointed.

The research was conducted in compliance with the Declaration of Helsinki (1975) which was revised in 1989 in Hong Kong.

From biochemical indicators in blood plasma the concentrations of total cholesterol (TC), α-lipoprotein cholesterol, β-lipoprotein cholesterol, glycosylated hemoglobin (HbAlc), glucose were measured. From apoptosis markers granzyme B was determined, from indicators of oxidative stress nitric oxide (NO) was measured, as well as cystatin C – as atherogenesis factor. Biochemical parameters were measured with the help of reagent sets produced by "Human" company (Germany), the contents of granzyme B and cystatin C – with the help of commercial sets produced by "USCN Life Science Inc" company (China), while nitric oxide concentration was determined using a commercial kit by

"R&D System". Statistical analysis was conducted with the help of Wilcoxon non-parametric criterion (Mann–Whitney test), differences were considered significant at *p <0,05; **p<0,01; *** p <0,001 compared to the control value.

Results and discussion

Biochemical data obtained in all studied groups are provided in table 1, while markers of apoptosis and oxidative stress are given in table 2.

As it can be observed, the amounts of lipoprotein complexes, triglycerides and free cholesterol are significantly different from the control group. Thus, in the control group the level of cholesterol was 2,85 ± 0,21 mmol/l, while the level of triglycerides was 1,01 ± 0,14 mmol/l. An increase in both indicators was identified during comparison of average values in patients with CHF and CHF with metabolic syndrome and diabetes mellitus type 2. Thus, concentration of cholesterol increased up to 4,51 ± 0,23 mmol/l (p <0,001) in I group, while in II and III groups – up to 5,25 ± 0,33 mmol/l and 5,47 ± 0,27 mmol/l, respectively. At the same time, the concentration of α-lipoprotein cholesterol declined by 36% in patients in I group, by 45% in patients in II group and by 61% in patients in III group. Along with this, β-lipoprotein cholesterol increased by 45% in patients in I group, by 64% in patients in II group and in average by 94% on patients in III group. Hypercholesterolemia and hyper-β-lipoproteinemia observed during studies contribute to the damage of the vascular endothelium as a result of atherogenesis. It was found that high density lipoproteins, a major anti-atherogenic fraction of lipoproteins, protect endothelial cells from apoptosis, therefore providing an important and new dimension of its anti-atherogenic activity [5].

Concentration of triglycerides in I group increased by 3,7 times, in II group – by 4,6 times and in III group – by 4,1 times, which is consistent with changes in carbohydrate metabolism, namely glucose and glycosylated hemoglobin. In CHF with DM-2 the content of glucose reached 7,3 ± 1,1 mmol/l, while in II group this indicator stood at 5,1 ± 1,2 mmol/l, in III group – at 4,1 ± 0,1 mmol/l. The level of Hв A1 in CHF with DM-2 constituted 6,7 ± 0,07%, while in I and III groups this indicator was nearly the same and on average stood at 5,4 ± 0,01 %.

Thereby, quantitative imbalance between parameters of lipid metabolism and lipoprotein complexes, as well as dynamics of changes in levels of glucose and glycosylated hemoglobin show similar trend in all studied groups of patients with CHF. Similar results were obtained in the analysis of the average values of nitric oxide and granzyme B in patients with CHF (Table 2).

While the control value of nitric oxide was 10,2 ± 0,4 μmol/l, in all studied groups this parameter increased to the following values: 13,7 ± 0,4 μmol/l in I group, 23,4 ± 0,5 μmol/l in II group, 21,4 ± 0,5 μmol/l in III group.

NO is a short-living molecule which breaks down within 6-30 seconds. At the same time, NO is recovered by its involvement in dinitrosyl iron complexes with thiol ligands or in 8-nitrozols, which may later gradually release NO. Such NO-containing complexes are formed in the tissues of physiologically active depot. Deposition of NO in the arterial wall begins with any increase of NO levels in the body, regardless of its cause. Excess amount of NO at first performs a compensatory function, aimed at improving the tissue perfusion. Later on, there is a transformation of reaction into the pathological one with the induction of apoptosis, activation of oxidative stress, destructive processes, increasing myocardial dysfunction [6, 7].

Nitric oxide is irreversibly inactivated by the reaction with hemoglobin (in oxygenated and dioxygenated forms) in the lumen of the blood vessel, by superoxide radical in the blood vessel wall or by oxygen in free solution [8, 9]. Reaction of nitric oxide with oxygen is accompanied with the formation of stable end products – nitrite and nitrate, which are indirect markers of concentration of nitric oxide in the body [10].

Cathepsins are direct executors of apoptosis, which is based on proteolysis caused by cysteine proteases such as caspases, cathepsins, granzyme B [11].

Although the content of granzyme B is widely studied in immunological disorders, the role of granzyme B/perforin system in cardiovascular pathology is insufficiently studied [12, 13].

In studied patients concentration of granzyme B significantly increased in I group by 1,5 times (p<0,001), in II group by 2,5 times (p<0,001), in III group – by nearly 2 times (p<0,001) in comparison with the control group.

Granzymes enter the target cell through pores formed by perforin and trigger apoptosis by activating caspases. Granzymes belong to the family of exogenous serine proteases. To date, five classes of granzymes are identified, such as A, B, H, K and M. These granzymes are produced in an inactive form and are activated by cathepsin C-mediated removal of the propeptide.

Granzymes A and B are the most studied ones. Granzyme B cleaves Asp or Glu residue of pro-caspases, resulting in activated caspase cascade, which ultimately leads to the death of the target cell. Activated caspases induce DNA fragmentation and cell apoptosis [14, 15]. Under normal conditions, the system granzyme B/perforin plays an important role in liquidation of abnormal cells, having antiviral activity and participating in the elimination of tumor cells. Thus, granzyme plays an important role in CTL-mediated immune response.

Table 1

Biochemical parameters in patients with CHF, M ± m

Parameters	Control group (N=10)	I group (N=41)	II group (N=39)	III group (N=34)
Cholesterol, mmol/l	2,85 ± 0,21	4,51 ± 0,23***	5,25 ± 0,33***	5,47 ± 0,27***
Triglycerides, mmol/l	1,01 ± 0,14	3,74 ± 0,37***	4,66 ± 0,24***	4,19 ± 0,29***
α-LP, mmol/l	1,29 ± 0,16	0,83 ± 0,11*	0,72 ± 0,08**	0,50 ± 0,03***
β-LP, mmol/l	2,49 ± 0,21	3,60 ± 0,26**	4,06 ± 0,35**	4,83 ± 0,18***
Glucose, mmol/l	4,2 ± 0,2	5,1 ± 1,28*	7,3 ± 1,1**	4,1 ± 0,1
Hв A1, %	5,5 ± 0,08	5,3 ± 0,01	6,7 ± 0,07*	5,4 ± 0,01
Cystatin C, mg/l	0,8 ± 0,01	1,1 ± 0,04	1,4 ± 0,07*	1,2 ± 0,06

*p <0,05; **p<0,01; ***p <0,001 compared to the control value

Table 2

Markers of apoptosis in patients with CHF, M ± m

Parameters	Control group (N=10)	I group (N=41)	II group (N=39)	III group (N=34)
NO, μmol/l	10,2 ± 0,4	13,7 ± 0,4***	23,4 ± 0,5***	21,4 ± 0,5***
Granzyme B, ng/ml	14,5 ± 0,9	22,0 ± 1,4***	36,4 ± 0,8***	28,8 ± 1,1***

*p <0,05; **p<0,01; *** p <0,001 compared to the control value

A number of researchers have suggested that granzyme B can cleave extracellular matrix [16]. It is assumed that granzyme B is involved in chronic, as well as acute inflammation in the atherosclerotic processes in coronary artery. It can be assumed that the inhibition of granzyme B can introduce a new therapeutic approach to the treatment of cardiovascular diseases and such conditions as prevention of progression of atherosclerosis, plaque rupture.

To characterize CHF complications we investigated the levels of cystatin C and found that the largest increase was observed in II group (by 1,8 times), while in I and III groups this indicator increased by 1,4 and 1,5 times, respectively. The literature shows that increased levels of cystatin C, regardless of other factors, are associated with the severity of induced ischemia [17, 18]. Normally cystatin C, as an inhibitor of cysteine proteinases, prevents the development of atherosclerotic lesions. However, increased level of cystatin C in atherosclerosis may serve as an evidence of large atherosclerotic plaques [19, 20]. Cystatin C in patients with DM-2 can serve as a reliable predictor of development of cardiovascular complications. For instance, it allows to predict the occurrence of arterial hypertension in patients with DM-2 and to some extent to measure the degree of progression of coronary atherosclerosis in these patients [21, 22].

Thus, it can be concluded that the studied parameters of oxidative stress and apoptosis, either individually or in combination, determine the degree of progression of CHF, can serve as markers of complications, and are correlated with biochemical changes in parameters of lipid and carbohydrate metabolism.

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Argumentation of the strategic directions for the development of pharmaceutical system in the Republic of Moldova

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Abstract

Background: To assess the actual pharmaceutical system in Moldova in order to show the strategic directions of the development of this system.
Material and methods: The methodology of the study is based on the systemic approach. In order to highlight the issues of Pharmaceutical system that are needed to be reflected in the development strategy, it has been elaborated a special method of questionnaires for selected specialists of the highest level of professional competence as respondents.
Results: All respondents mentioned that having a development strategy for the pharmaceutical system is a real necessity. There were determined 10 directions of the development strategy and the importance of each direction. Also, the respondents proposed additionally 21 directions and 21 another real problems to include in the strategy.
Conclusions: It has been highlighted the absence of the development strategy of the pharmaceutical system in the Republic of Moldova, which will bring to the light systematically full range of pending issues. It has been developed a special method of questionnaires for selected specialists of the highest level of professional competence taking into consideration 3 factors: theoretical and practical training, seniority and experience of elaborating legislative and normative acts in the field of medicines and pharmaceutical activity. There were argued five strategic directions of the development of pharmaceutical system in the Republic of Moldova, which include themselves about 50 issues, some more general, others – concrete, but all are towards achieving the overall goal – providing benefits to public and individual health.

Key words: pharmaceutical system, development strategy.

Introduction

The need for a development strategy of any system, especially a system involving human and his health is imperative and it is out of discussion. One of the principles of the systemic approach is the "strategic development", which demonstrates once again the need for elaboration of the development strategy for pharmaceutical system in the Republic of Moldova.

Some motivational underlines regarding the need to develop a strategy for pharmaceutical system in the Republic of Moldova were mentioned about 7 years ago – at the 6th Congress of Pharmacists [1].

It should be noted that National Health Politics [2] addresses only two aspects of the pharmaceutical system – "The implementation of computerized technology in the health domain will provide the centralized evidence of drugs on the market and will contribute to the security of pharmaceutical products. The population will be guaranteed access to essential and qualitative medicines". It is obvious that these two issues do not cover all the problems facing today's pharmaceutical system.

The development strategy of the health system for the period 2008-2017 [3], as well, does not provide the development of pharmaceutical systems using systemic approach.

In this strategy, the drug and pharmaceutical activity domain is included in section 4 "Resource Management" and section 70 "The rational management of drugs", which provide measures in two directions:

a) Ensure pharmaceutical safety;
b) Ensure accessibility to the drugs.

Ensuring the security of pharmaceutical system is expected to be achieved through series of concrete activities:

- the development and implementation of best practice rules – GLP, GCP, GMP, GDP, GPP;
- the improvement of computerized technology system in order to record the drugs circulation;
- the development of the mechanisms which will ensure the rational use of medicines.

In order to ensure accessibility to medicines there were provided the following activities:

- to reorganize the pharmaceutical departments in the Centers of General Physicians (GP's);
- to expand the compensated drugs assortment and the improvement of the mechanisms in order to ensure the population with these drugs;
- to improve the mechanism of price formation for drugs;
- to stimulate the development of domestic pharmaceutical industry.

The mentioned strategic directions are still actual but the current situation of the pharmaceutical system is affected by the multitude of issues that need to be made as strategic objectives in a new term development program.

At the end of 2003 the Government approves national public health strategy for 2004-2020 [4], but in this strategy the words «drug» or «pharmaceutical» have not been found.

This strategy will not substitute any State Policy in the domain of Medicines [5], because this policy document

requires be reviewed and updated in accordance with the situation that it is created in the Moldovan pharmaceutical system [6].

The purpose of this document is to assess the actual pharmaceutical system in Moldova which is oriented to argue the strategic directions of the development of this system.

Material and methods

With the purpose to assess the situation on strategic planning in the pharmaceutical system of Moldova there was started implementing of a special questionnaire in this regard.

Special interviewing is a method of sociological research in the process of which the respondents are experts selected on the principle of the highest level of professional competence.

In order to determine the competence of respondents, there was developed an algorithm, which includes three basic criteria:

I. **Theoretical and practical competence (P_{tp}):**

- having degree in pharmacy and work experience at least for 2 years – 2 points;
- theoretical work (published scientific articles) and practical activities, the 1st or the 2nd professional category – 5 points;
- PhD degree in pharmacy and practical activity, the highest professional category - 10 points;
- Work experience (V_m):
- Work experience up to 5 years – 2 points;
- Work experience >5 years to 10 years – 5 points;
- Work experience >10 years to 15 years – 8 points;
- Work experience over 15 years – 10 points;

II. **Experience in elaborating of legislative and normative acts in the domain of medicines and pharmaceutical activity (E_{an}):**

- Underlegislative acts/projects – 2 points;
- Underlegislative acts/projects and projects of Government decisions – 5 points;
- Underlegislative acts/projects and projects of Government decisions and Orders – 8 points;
- Underlegislative acts/projects and projects of Government decisions, projects/orders and projects of politics documents – 10 points;

Using mentioned criterias in p. I-III there was elaborated the following algorithm:

$$k_i = \frac{1}{10}\left(\frac{Ptp+Vm+Ean}{3}\right), \text{ in which} \quad (1)$$

k_i – integrated coefficient characterizing the degree of responsibility of the respondent – the expert.

In order to validate the developed algorithm, there were determined the minimum and the maximum limits;

Minimum limit: $$k_{i\,(min)} = \frac{1}{10}\left(\frac{2+2+2}{3}\right) = 0{,}2 \quad (2)$$

Maximum limit:

$$k_{i\,(max)} = \frac{1}{10}\left(\frac{10+10+10}{3}\right) = 1{,}0 \quad (3)$$

The acceptable limit: $(k_{i(min)} + k_{i(max)}): 2 = 0{,}6$.

Thus, in the questionnaires should be involved respondents- experts possessing the degree of competence within 0,6 – 1.0.

To collect the information on strategic planning in the pharmaceutical system there was developed a questionnaire (fig. 1).

All in total, 40 questionnaires there were printed and distributed, but there were collected only 36 or 90% of the required number.

Results and discussions

Characteristics of the respondents

In the process of questionnaires, there were involved 38 pharmacists, the competence of which was illustrated by the integrated coefficient (ki), which was calculated using formula (1). The results are presented in the table 1.

Table 1

Assessment of the respondents' competence

Number of questionnaire	Integrated coefficient of competence (k_i)	Number of questionnaire	Integrated coefficient of competence (k_i)	Number of questionnaire	Integrated coefficient of competence (k_i)
1	**0,46**	13	**0,6**	25	**0,93**
2	**1,0**	14	**0,66**	26	**1,0**
3	**0,66**	15	**0,93**	27	**0,76**
4	**0,6**	16	**0,93**	28	**0,7**
5	**0,76**	17	**0,83**	29	**0,66**
6	**0,6**	18	**0,83**	30	**0,66**
7	**0,76**	19	**0,83**	31	**0,76**
8	**0,66**	20	**0,83**	32	0,2
9	**0,66**	21	**0,76**	33	**0,6**
10	**0,66**	22	**0,66**	34	**0,83**
11	**0,76**	23	**0,66**	35	**0,66**
12	**0,93**	24	**0,66**	36	**0,66**

As in the process of questionnaires should be involved experts – respondents possessing a degree of competence within [3]0,6-1.0 from the total of 36 questionnaires, two of questionnaires were excluded: questionnaire no. 1 (ki = 0.46) and questionnaire no. 32 (ki = 0.2). Thus, in the analysis of the information on strategic planning in the pharmaceutical system were included 34 questionnaires of pharmacists respondents.

According to domain of activity of the pharmacists respondents, they work in different pharmaceutical fields: 35.29% – Pharmacists, chiefs of pharmaceutical companies (12 respondents); 23.52% – Instructors of the pharmacists

Cassette 1

Questionnaire

Dear colleague, the absence in Moldova of a strategy for development of pharmaceutical system very often led to chaotic actions that keep this system in a «feverish» stage.

To redress the situation, please answer the questions of this questionnaire.

1. Do you consider it necessary to have a strategy for development of Moldova's pharmaceutical system?
- □ Yes
- □ No

2. Motivate the negative answer (by tick);
- □ is it sufficient to have a strategic development of the health system;
- □ is it sufficient the existence of State Policy in the domain of Medicine;
- □ the main components of the pharmaceutical system are private and they do not need „the state strategy";
- □ other reasons (specify them): _____

3. Motivate the positive answer (by tick);
- □ the strategy and strategic planning is the most effective way of selecting their aims, objectives and development programs;
- □ the strategy and the strategic plan is a real support for short - term planning and operational phase;
- □ the strategy of development of the health system does not contain 0,1 part of the strategic issues of the pharmaceutical system;
- □ other reasons (specify them): _____

4. Set the rank (numbering taking into consideration the priority) for the next 10 points which are planned to be included in the strategy of development of the pharmaceutical system of RM (1-most important... 10 the least important)
- □ Improving the undergraduate and postgraduate training of pharmacists.
- □ The quality of pharmaceutical services provided in outpatient clinics and hospitals-based on ethic and professional principles.
- □ Authorization of pharmaceutical activity (licensing and accreditation).
- □ Pharmaceutical Assistance in rural areas.
- □ Ensuring patients with compensated drugs in outpatient departments.
- □ Improving the mechanism of price formation for drugs.
- □ Resumption and further development of medicines preparation.
- □ Improving the mechanisms of ethical promotion of medicines.
- □ Solving the situation regarding to informational ensuring of pharmaceutical act.
- □ Promoting the concept of good governance of the pharmaceutical system.

5. From your point of view, please mention additional development directions that deserve to be included in the strategy and determine their priority (points 1 to 10):

5.1. _____
5.2. _____
5.3. _____
5.4. _____
5.5. _____

6. From your point of view, please mention at least five of the most important and concrete issues that should be included in the strategy of the development of the pharmaceutical system:

6.1. _____
6.2. _____
6.3. _____
6.4. _____
6.5. _____

7. From your point of view, which would be the optimal period of the strategy of the development of the pharmaceutical system?

• 5 years; • 7 years; • 10 years; • 15 years; • 20 years.

8. Please specify some personal data (by tick):

a) Domain of activity;
- • Pharmacist, chief of a pharmaceutical company; • Pharmacist practitioner of a pharmaceutical company;
- • Training the pharmacists; • pharmaceutical research;
- • coordination and control system; • others

b) Work experience (tick):
- • up to 5 years; • >5-10 years; • >10-15 years; • >15 years;

c) Professional category that you hold at the moment (tick):
- • 2nd; • first ; • the highest;

d) Scientific degree (tick):
- • habilitated doctor in pharmaceutical science; • doctor in pharmaceutical science; • no scientific degree;

e) Have you experience in elaborating of the project of documents (tick)
- • sublegislative • yes • no
- • government decisions • yes • no
- • Orders • yes • no
- • politics • yes • no

Thank you for your support! _____

(sign) - optional

Fig. 1. Questionnaire Cassette 1.

(8 respondents); 23.52% – Pharmacist practitioners of pharmaceutical companies (8 respondents); 11.76% – coordination and control system (4 respondents); 5.88% – pharmaceutical research (2 respondents);

According to work experience in the domain there were obtained the following data: 5 to 10 years – 5.88% (2 respondents); > 10 to 15 years – 14.7% (5 respondents); > more than 15 years – 79.41%.

Regarding the professional category, most of the specialists have the highest category – 79.41%, the 1st category – 14.7% and 5.88% have the 2nd category.

According to scientific degree the pharmacists are classified as follows: 17.64% are doctors in pharmaceutical sciences and 2.94% – pharmacists with habilitated doctor in Pharmaceutical Sciences, the rest of the respondents (79.41%) have no scientific degree.

Experience of elaborating normative legal acts possess 12 respondents (35.29%), including trainers of pharmacists – 17.64%, specialists of coordination and control system – 11.76% and pharmaceutical research – 5.88%.

Analysis of answers

When asked whether there is a need to create a strategy for the development of the pharmaceutical system in Moldova all 34 pharmacists – respondents gave an affirmative answer. As a motivation for their positive response were ticked the following: 41.37% – mentioned p. a) – the strategy and strategic planning is the most effective way of selecting their aims, objectives and development programs; 31,03% – mentioned p.b) – the strategy and the strategic plan is a real support for short - term planning and operational phase; the last place 27,58% – ticked p.c) – the strategy of development of the health system does not contain 0,1 part of the strategic issues of the pharmaceutical system.

As a result of quantifying the strategic directions by establishing of the rank, it was determined the importance of each direction and it was highlighted the priority of the strategic directions in relation to the deadline needed to achieve the stipulated plan for the strategy (tab. 2).

All the experts added to the proposed directions in the 4th question considered to indicate some other directions that might also be included in the strategy for the development of the pharmaceutical system. Totally they proposed 21 more directions, including: 5 more directions were proposed by one expert; the other expert came with 4 more proposals; the other 5 experts came with 3 proposals; 13 experts came with 2 proposals and 4 experts gave one proposal each (tab. 3).

In conclusion, it was obtained that the first three strategic direction proposals were formulated by five experts; the following five proposals were submitted by four experts; next 5 - by 3 experts, following six proposals were formulated by 2 experts and the last two directions were formulated only by one expert. Thus, the degree of repeatability of other strategic directions which were proposed by the pharmacists-experts is 90.5%.

To integrate the responses of the experts to question 5 taking into consideration the directions formulated in question 4, it was proposed to formulate question 6 – to mention at least 5 of the most pressing and important issues that should be included in the strategy of the development of the pharmaceutical system. The answers to the 6th question are shown in table 4.

From the total number of respondents – experts, 32 of them mentioned 5 important issues, and 2 experts mentioned 4 issues each. Thus, all pharmacists-experts have mentioned 168 issues, which are oriented in two directions. The medium repeatability degree is 8, maximum – 16 and minimum – 2. From the total number of urgent issues, 3 of them were proposed by 16 respondents, 5 – by 14 respondents, 12 – by 4 respondents and only one issue was proposed by 2 experts.

On the 7th question (the optimal period for the strategy),

Table 2

Ranking according to priorities of strategic directions of the development of the Republic of Moldova pharmaceutical system

Rank	Strategic direction	Accumulated amount
1	Authorization of pharmaceutical activity (licensing and accreditation)	103
2	The quality of pharmaceutical services provided in outpatient clinics and hospitals-based ethic and professional principles	105
3	Improving the mechanism of price formation for drugs	146
4	Pharmaceutical assistance in rural areas	156
5	Promoting the concept of good governance of the pharmaceutical system	164
6	Ensuring patients with compensated drugs in outpatient departments.	183
7	Improving the undergraduate and postgraduate training of pharmacists	197
8	Improving the mechanisms of ethical promotion of medicines	214
9	Resumption and further development of medicines preparation	220
10	Solving the situation regarding informational ensuring of pharmaceutical act	267

the answers of the pharmacists-respondents were: 5years – 11 (32.35%); 7 years – 4 (11.77%); 10 years – 17 (50%); 20 years 2 (5.88%). The 15 year period was not mentioned by any expert. Thus, it is recommended the period of 10 years (2017-2027) for the strategy of the development of the pharmaceutical system in Moldova.

According to the survey, the data from the tables 2-4 were grouped into the following five complex strategic directions, in which are elucidated all the problems and issues:

I – Promoting an ethical pharmacy;

II – Strengthening the quality of the pharmaceutical activity;

III – Ensuring of the accessibility to the medicines;

IV – Ensuring good conditions for the development of pharmaceuticals units;

V – Reviewing the pharmaceutical legislation by harmonization with the EU acquis.

Table 3

Other directions that deserve to be included in the strategy according to the respondents' opinion

Nr. d/r	Proposed directions	Nr. of the experts
1	Promoting of an ethical pharmacy	5
2	Rational use of medicines	5
3	Compliance with the regulation regarding expansion of the pharmacies	5
4	Licensing of the pharmaceutical activity by the Ministry of Health	4
5	Establishing a more drastic legislative regulating framework of the pharmaceutical activity and improving the legal framework	4
6	Extending the number of pharmacies with medicines preparation	4
7	Pharmacies should belong only to pharmacists	4
8	Excluding the commercial interest in pharmacies	4
9	To revise the regulations and standards that directly and indirectly affect the pharmaceutical system in the Republic of Moldova	3
10	Managing the pharmacies and pharmaceutical warehouses only by specialists with pharmaceutical education	3
11	The development and implementation of best practice rules (the ones that are not elaborated yet)	3
12	Strict compliance with occupied positions in the institutions according to education and skills of the pharmacists.	3
13	To restore the collaboration between the physician and pharmacist	3
14	More active promotion of healthy lifestyle oriented towards disease prevention	2
15	To create and promote the concept of the pharmacy "model" by establishing advanced standards to all aspects of pharmaceutical activity	2
16	Reforming of the former central pharmacies into multifunctional units, including coordination and control.	2
17	Restoring a pharmacy chain with public capital	2
18	Taking over Romanian legislation in regulating pharmaceutical activity	2
19	Implementing informational system regarding patients files records	2
20	Improving the situation regarding human resources in pharmaceutical system (recording, migration, education recognition, equivalence of diplomas)	1
21	Creating database for pharmacists and their subsequent record, in order to prevent practicing pharmaceutical activity by non-professional people	1

Table 4

The most pressing and important issues that should be included in the strategy of the development of the pharmaceutical system

Nr. d/r	Most pressing and important issues	Nr. of the experts
1	Licensing and accreditation	16
2	Compliance with regulation on pharmacies location	16
3	Taking concrete and effective measures that would not allow practicing pharmaceutical activity by non-specialists	16
4	Perfecting pricing system for medicines	14
5	Preventing the pharmaceutical warehouses from holding pharmacies (important factor in the exaggerated development of pharmaceutical chains)	14

Nr. d/r	Most pressing and important issues	Nr. of the experts
6	Reorganizing of Pharmacists Association activity with an orientation to College of Pharmacists from Romania	14
7	Returning to actuality presence and functionality of automated information system of recording the movement of drugs	14
8	Approving the plan of the development of the pharmaceutical system	14
9	Pharmacists technician should not be allowed to dispense Rx products	4
10	Ensuring physical accessibility of medicines	4
11	Reviewing the right of existence of pharmacy chains	4
12	Orientation to recognized providers of medicines and exclusion of dubious suppliers	4
13	Liquidation of community pharmacy chains	4
14	Restoring the legislative framework regarding the statistics in the pharmaceutical domain	4
15	Proper hospitals supply with necessary qualitative and harmless medicines	4
16	Development of best practice rules	4
17	Correct prescribing the medicines by the doctors	4
18	Fight against abuse of drug advertising	4
19	Excluding the corruption in the process of centralized drug acquisitions	4
20	The development of domestic pharmaceutical industry	4
21	Changes / completing of the Administrative Code	2

Conclusions

1. It has been highlighted the absence of the strategy of the development of Moldovan pharmaceutical system, which would take care systemically of the full range of pending issues.

2. It has been developed a special method of questionnaires in which were involved specialists-experts with a quantified by three – factor degree of competence: theoretical and practical confidence, work experience and experience in elaborating of legislative and normative regulations in the domain of medicines and pharmaceutical activity.

3. There were highlighted five strategic directions for the development of Moldovan pharmaceutical system, which include about 50 issues, some of them are more general, others – are concrete, but all of them are oriented towards achieving a single goal – providing benefits to public and individual health.

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Experimental study of new 3-(2-R^1-6-R^2-4-oxyquinoline-3(4H)-yl)alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound)

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Abstract

Background: Screening studies have revealed in new 3-(2-R1-6-R2-4-oxyquinoline-3(4H)-yl)alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound) expressive analgesic properties without any damaging effects on the stomach. Therefore, in-depth study of the pharmacological properties of PC-66 compound as a pain management agent is considered topical. Objective of the study – to evaluate a pain-killing effect of PC-66 compound compared to ketorolac and diclofenac sodium on various rat pain models.

Material and methods: In experiments on 101 Wistar male rats (180-210 g) of somatic model (tail-flick) and neuropathic pain model (ligation of the sciatic nerve), and formalin test (5% formalin solution, 0.1 ml subplantarly) we investigated the antinociceptive activity of the PC-66 compound (1.0 mg/kg) versus ketorolac (2.4 mg/kg) and diclofenac (4.0 mg/kg) administered intraperitoneally.

Results: In the tail-flick model, PC-66 compound presented significant growth of PT at Hour 1 and Hour 2 by 40.6% and 50.6%, respectively. The analgesia effect of the test compound was superior to the one of diclofenac sodium, but inferior to ketorolac at Hour 1 and Hour 2, yet surpassed it by duration of action. In the formalin test model, analgesic effect of compound PC-66 was the most evident in the first (central) phase, and slightly changed the latent period and duration of the second phase of the test, while diclofenac mostly influenced Phase II (inflammatory) of the formalin test. In the model of neuropathic pain, compound PC-66 also demonstrated pronounced pain-killing effect: PT of subject rat limb grew on average by 46.7% in 2 hours following intraperitoneal administration. For this activity, PC-66 was slightly inferior to ketorolac, which caused PT growth by 51.9%.

Conclusions: new 3-(2-R1-6-R2-4-oxyquinoline-3(4H)-yl) alkyl (alkaryl-, aryl) carboxylic acid derivative (PC-66 compound) presented distinct analgesic effect both in somatic and neuropathic pain models.

Key words: carboxylic acid derivative (PC-66 compound), analgesic effect, rat pain models.

Introduction

Search for and development of highly efficient and safe medicines for management of pain of various natures is still one of the priorities of modern pharmacology [1, 2]. 4-oxo (amino-) quinazoline derivatives have proved to be quite promising among the classes of chemicals. According to the research literature [3, 4], this class of compounds have shown cerebro- and actoprotective and antinociceptive properties, and appeared to be low-toxic substances [5]. In previous screening studies of pain models caused by electrical (pulse) stimulation of rectal mucosa and thermal stimulation of rat tail, we found two leading compounds (PC-66 and PC-199) with pronounced analgesic effect within a range of new 4-oxo (amino-) quinazolin derivatives, which appeared to be equivalent to diclofenac sodium and ketorolac both in the efficacy and effect duration [6]. However, the PC-66 compound revealed to be much safer for the gastric mucosa than PC-199 [7]. This became the basis for further in-depth study of the pharmacological properties of PC-66 compound as an anesthetic medicine.

Study objective – to evaluate the analgesic effect of the above substance compared with ketorolac and diclofenac sodium in various rat pain models.

Material and methods

Experiments were conducted on 101 male 180-210-gram Wistar rats bred at vivarium of DU 'Instytut farmakolohii ta toksykolohii NAMN Ukrainy' (State institution "Institute of Pharmacology and Toxicology of AMS of Ukraine"), kept in standard conditions of vivarium on traditional diet with 12 –hour light-and-dark regime and access to water ad libitum. All experiments were carried out in compliance with the required bioethical standards. The following nociception models were used in the study: thermal stimulation model (tail-flick) for registration of changes in the latent period of tail flicks against the focused beam of light (pain threshold, PT) measured in seconds [9]; comparative benchmarks for duration of latent period and its changes in 1, 2, 4 and 6 hours after administration of test compound and reference medicine; and the model of neuropathic pain after ligation of the sciatic nerve [9]. The intensity of mechanical hyperalgesia was evaluated on Day 14 after surgery using Dolorimeter Baseline, USA. The pain threshold (PT) in subject and intact limbs was taken as minimal pressure on rat foot (g/mm^2), which caused painful reaction in animals (vocalization and/or foot flick). More information about central and peripheral components of analgesic response was obtained after the formalin test (5% formalin solution, 0.1 ml subplantarly). The monitoring lasted 60 minutes after algogenic substance administration. We registered I and II phase latent period duration, and the total duration of pain in each phase of the test. Single dose of PC-66 compound, ketorolac and diclofenac were administered intraperitoneally. Animals in the control group received equivolumic quantities of solvents. The results were processed by methods of variation statistics using STATISTICA 8.0 software and nonparametric methods of analysis [10].

Results and discussion

The results of screening studies of PC-66 compound and reference medicines allowed us to define conditionally-effective antinociceptive doses of these substances administered intraperitoneally, which were 1.0, 2.4, and 4.0 mg/kg for PC-66, ketorolac and diclofenac sodium, respectively. Therefore, these same doses of studied substances were used for further study of analgesic effect.

The first phase of thermal stimulation test (tail-flick) investigated the extent and duration of PC-66 effect compared to ketorolac and diclofenac sodium. The results presented presumable overrun of rat PT with quinazoline derivative well in an hour after administration (40.6%). Maximum growth of antinociceptive effect was registered at Hour 2 (+ 50.6%), which decreased gradually and at Hour 6 was 20.4% compared to the baseline (fig. 1). The analgesia effect of the test compound was found superior to diclofenac at all times of the study, but inferior to ketorolac at Hour 1 and 2 after administration, yet exceeding the duration of the above effect (at Hour 6, the PT growth influenced by PC-66 was 20.4%, versus 8.93% influenced by ketorolac).

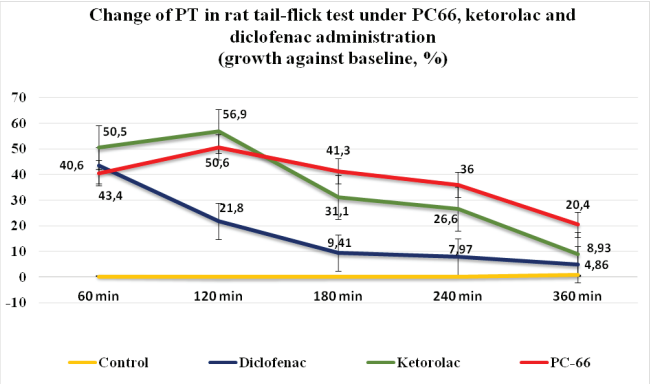

Fig. 1. Behaviour of analgesic effect of PC-66, ketorolac and diclofenac sodium administered intraperitoneally at suppositive – effective doses.

The obtained data led to further in-depth studies of analgesic effect in formalin test on rats, which allowed distinguishing central and peripheral components of antinociceptive effect in more details. The results showed that subplantar administration of formalin caused pain reaction in intact animals that started in average 0.69 ± 0.36 minutes following the procedure. Pain reaction manifested in raising and swinging the subject leg (Phase 1), as well as in biting and licking (Phase 2). Total duration of pain was 5.02 ± 0.42 minutes. After that, the animals calmed down, and about 20 minutes did not present any change in behavior. Then again the signs of pain reaction returned, clinically manifested in two phases with total duration of 31.9 ± 2.36 minutes. At the end of the monitoring period (60 minutes), signs of pain in considerable number of rats did not abate. The animals, intraperitoneally administered solvents (control group) instead of study medicines, presented all above-mentioned clinical signs of pain reaction, not different in severity and duration of Phase I and II latent periods from intact animals (tab. 1).

Table 1

Analgesic activity of PC-66 (1 mg/kg IP) and diclofenac (4 mg/kg IP) in rat model formalin test (M ± m, n = 10)

Group of animals	Phase I		Phase II	
	Latent period, min	Phase duration, min	Latent period, min	Phase duration, min
Intact animals	1.69±0.36	5.02±0.45	21.3±2.45	31.9±2.36
Control (solvent)	1,67±0.29	5.14±0.61	21.2±1.21	32.0±1.09
Diclofenac	1.79±0.16	5.64±0.28	27.3±2.19*	18.7±2.58*
PC-66	2,41±0,28#	4,48±0,29#	22,2±1,04#	29,4±1,15#

Notes: * – statistically significant differences against the control ($p<0.05$), # – statistically significant differences against diclofenac ($p<0.05$).

Instead, administration of PC-66 and diclofenac sodium, significantly changed pain behavior of animals. PC-66 significantly (44.3%) extended the duration of latent period of phase I in formalin test and statistically significantly (12.8%) reduced the duration of this phase, which substantionally characterized central mechanisms of antinociceptive action. Unlike PC-66, diclofenac sodium practically did not influence the duration of this phase. Having analyzed the influence of PC-66 and diclofenac on phase II of the formalin test, you can see that the tested compound influenced the duration of the inflammatory phase much weaker than diclofenac. Thus, the increase in average latency period and reduction of the total duration of pain reaction changed insignificantly (4.7 and 8.1%, respectively), while the latent period influenced by diclofenac demonstrated statistically significant growth by 28.8%, while duration of pain decreased by 41.5% compared to control ($p<0.05$).

The received data showed that PC-66 demonstrated analgesic properties in the rat model formalin test, manifested mostly in the Phase I of the model pathology, which involved mainly central antinociceptive mechanisms and slightly reduced latent period and duration of phase II of the test. These properties were different from non-steroid anti-inflammatory effect of diclofenac sodium, which altered Phase II (inflammatory), which mechanisms were influenced by mediators of inflammatory response, mainly prostaglandins, leukotrienes, etc.

Another important aspect of analgesic action of biologically active compounds is their effect on the neuropathic pain model. According to the results of the study, on Day 14 after nerve ligation, animals developed a chronic pain syndrome manifested in behavioral reactions of rats and

Table 2

Analgesic activity of PC-66 compared to ketorolac under conditions of intraperitoneal administration in rat neuropathic pain model (M ± m, n = 7)

Groups	PT, g/mm²				
	Intact limb	Subject limb	Changes in pain perception threshold*	In 2 hours after compound administration	Changes in pain perception threshold**
Control	406.4±19.9	390.7±22.4	-3.86%	392.1±20.5	+0.35%
PC-66 1 mg/kg	380.7±20.4	298.6±19.7#	-21.5%	437.9±29.4&	+46.7%
Ketorolac 2.4 mg/kg	407.1±5.76	309.3±17.4#	-24.0%	470.0±24.1&	+51.9%

Notes: 1. Control – sham-operated animals that received equivolumic quantities of solvents; 2. * – compared to intact limb; 3. ** – compared to subject limb before compound administration; 4. # – statistically significant differences ($p<0.05$) compared to intact limb; 5. &- statistically significant differences ($p<0.05$) compared to subject limb before administration of compounds.

decrease of pain threshold in subject limbs by 21-24%, in average, compared to intact limbs (tab. 2). Under these conditions, PC-66 demonstrated expressed analgesic effect, as evidenced by increase of PT in subject limbs 2 hours after intraperitoneal administration by average 46.7%, compared with the index before administration of the substance. For this activity, PC-66 was slightly inferior to ketorolac, which presented PT growth by 51.9% ($p <0.05$).

Conclusions

Thus, we have received information that confirmed pretty high analgesic effect of new 3-(2-R^1-6-R^2-4-oxyquinoline-3(4*H*)-yl)alkyl-(alkaryl-, aryl-) derivative of carboxylic acid (PC-66 compound) at both somatic and neuropathic pain models. The antinociceptive efficacy of this compound was found similar to ketorolac and diclofenac ones, and even surpassed them in the activity. It is worthy to note that effect of the compound was the most evident in Phase I of the formalin test, while its influence on the second (inflammatory) phase was negligible. Having compared the actual findings with those from the previous studies, according to which the compound did not present any damaging effect on the stomach both in subchronic and chronic administration [7, 8], one may think of the lack of mechanisms of any significant effect on prostaglandin system and high probability of involvement of other antinociceptive mechanisms, which requires further research of the issue. Conducting in-depth studies of mechanisms of analgesic action of 4-oxo (amino-) quinazoline derivative (PC-66 compound) and its other effects may help provide possible indications for its use as a medicine with distinctive analgesic effect.

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Antimicrobial effect of wound healing nano-containing polymer materials

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Abstract

Background: Treatment of infected wounds presents the biggest difficulty for the surgeon. The treatment of skin lesions has recently increased due to the effect of developed local multi-application forms saturated with nano-sized antiseptic preparations, namely with metals nano-oxides: zinc oxide, magnesium oxide, iron oxide, silver oxide, etc.

Material and methods: We have developed a biodegradable polymer film that is flexible and has the ability to biodegrade and deliver the remedy to the area of injury. Saturated with active components, polymer films have been studied for their antimicrobial effect against clinical strains of opportunistic microorganisms commonly present in wounds, which were isolated from patients with purulent-septic diseases: *Staphyloccus aureus, Staphyloccus epidermidis, Staphyloccus haemolyticus, Streptococcus pyogenes, Escherichia coli, Citrobacter freundii, Pseudomonas aeruginosa* and *Candida tropicalis yeasts*.

Results: The films saturated with zinc nano-oxide have been characterized by high antimicrobial effect against all used microbial cultures. Films containing 5 and 10% of ZnO appeared to produce larger areas of growth inhibition against the most strains in comparison to the films containing 1% of ZnO. In contrast to zinc nano-oxide, the presence of magnesium in films hasn't proved to have any antimicrobial effect.

Conclusions: Biodegradable films with 5% of nano-oxide content have optimal antimicrobial effect in vitro against relatively opportunistic microorganisms and require further experimental and clinical studies.

Key words: wound healing polymer materials, zinc nano-oxide.

Introduction

The biggest difficulty for the surgeon is the treatment of infected wounds. The incidence of surgical infection in surgical diseases is not reducing and makes up 24-36% [1].

Recurring infectious diseases, as well as the continuous development of antibiotic resistance among different bacteria is a serious problem and danger to public health in the world [2].

Enterococci, Staphylococci, Streptococci and other pathogens cause a wide range of infectious diseases and result in surgical wounds infection and abscess that are very difficult to treat. Despite antimicrobial therapy, morbidity and mortality associated with the bacterial infection still remain high, partly due to the ability of these organisms to develop resistance to almost all antibiotics [3,4,5].

Nowadays, there are two main approaches to the purulent wounds treatment in the first phase of wound healing. The first one deals with finding the most effective ways of necrotic masses quick removing from the purulent wound (developing of experimental wound healing coatings to remove damaged tissue caused by different mechanical, thermal and other purulent-inflammatory or degenerative processes). The second one is based on the medications and tools development and their application that can limit and eliminate wound infections [6,7,8].

Improving local wound treatment is aimed primarily at the application of modern highly efficient drugs depending on the particular phase of wound healing [9,10]. Therefore, to solve these problems a new generation of drugs or agents should be used to combat bacterial infections in the wound. So, local multi-application forms with prolonged osmotic effect for the treatment of damaged skin have been developed and applied increasingly, to prevent drying of the wound, stimulate the growth of granulation and saturated antiseptic nano-sized drugs, namely metals nano-oxides: zinc oxide, magnesium oxide, iron oxide, silver oxide, etc. [11,12,13,14,15,16,17].

One of such promising antimicrobial agents in polymer materials that currently is intensively investigated, and has both anti-inflammatory and antiseptic effect is zinc nano-oxide (ZnO) [18,19,20].

The aim of the study was to investigate the antimicrobial properties of biodegradable wound healing polymer materials in the form of films with different concentrations of metals nano-oxides during the experiment in vitro.

Material and methods

We have developed a biodegradable polymer film [21] based on gelatin, polyvinyl alcohol (PVA), lactic acid, distilled water and glycerin, which are blended under the influence of microwave radiation, and which is flexible and has the ability to biodegrade and deliver the remedy to the area of injury. There was synthesized a polymer base by the method optimized previously (sample №8) and there was also synthesized a base saturated with nano-oxides of zinc (samples №1, 2, 3), of magnesium (samples №4, 5, 6) at concentrations (1%, 5% and 10%) respectively, and also saturated with common antiseptic decamethoxinum (sample №7) (tab.1)

Saturated with active components polymer films have been studied for their antimicrobial effect against clinical strains of opportunistic microorganisms commonly present in wounds, which were isolated from patients with purulent-septic diseases: *Staphyloccus aureus, Staphyloccus epidermidis, Staphyloccus haemolyticus, Streptococcus pyogenes, Escherichia coli, Citrobacter freundii, Pseudomonas aeruginosa* and *Candida tropicalis yeasts*.

Identification of microorganisms clinical strains was based on morphological and cultural properties in accordance with the recommendations of the 9th edition of 'Bergey's Manual of Determinative Bacteriology'[22] and biochemical microtests sets 'STAPHYtest 16', 'STREPTOtest 16', 'ENTEROtest 24', 'NEFERMtest 24' (Lachema, Czech Republic).

Staphylococcus test strains differed with their antibiotic

Table 1

Polymer films compositional analysis

Sample №	Gelatin, g	PVA, g	Water, ml	Lactic acid, ml	Glycerin, ml	Decame-thoxi-num, ml	Nano-oxides	
							mg	%
1	2	1	15	0.05	0.02	0	0.025	1% ZnO
2	2	1	15	0.05	0.02	0	0.125	5% ZnO
3	2	1	15	0.05	0.02	0	0.25	10% ZnO
4	2	1	15	0.05	0.02	0	0.25	1% MgO
5	2	1	15	0.05	0.02	0	0.125	5% MgO
6	2	1	15	0.05	0.02	0	0.25	10% MgO
7	2	1	0	0.05	0.02	15	0	0
8	2	1	15	0.05	0.02	0	0	0

resistance: there were used methicillin-resistant and methicillin-susceptible strains in research. Methicillin-resistance of *S. aureus* and *S. haemolyticus* strains was proved with positive latex agglutination reaction to penicillin-binding protein PBP2 (Slidex® MRSA Detection, bioMerieux, France). Both methicillin-resistant strains of staphylococci are also characterized by the associated resistance to macrolides, tetracyclines, aminoglycosides and fluoroquinolones. Culture of *E. coli* proved to be sensitive to antibiotics, including aminopenitsyline, cephalosporins and carbapenems.

Other used in research Gram-negative bacteria are β-lactamase producers of extended range (ESβL). β-lactamase activity was detected on the comparative basis of strains sensitivity to cefoperazone and to combinations of tsefoperezon / sulbactam. The strain of *Candida tropicalis* showed weak sensitivity to polyenes (nystatin, amphotericin B) and alilamines (terbinafine) in a dose-dependent sensitivity to imidazole (especially ketoconazole) and triazoles (fluconazole, itraconazole, voriconazole).

Microbial cultures were grown in liquid nutrient medium for 24 hours. Then 1 ml of daily microbial culture was diluted with isotonic sodium chloride solution at the ratio of 1: 1000. The obtained suspension was planted into the elective nutrient media prepared by 'a spread bacterial lawn' approach.

Determination of films antibacterial properties was conducted by disk-diffussion method. 6 mm diameter discs made from films samples were applied on the surface of agar which was planted smoothly with standardized test cultures suspensions. Experiments results were calculated after plating incubation in the thermoregulator for 24 hours. The obtained digital images on the plates were processed with the help of a computer program UTHSCSA ImageTool 2.0 [23].

Dimeters of microorganisms inhibition zones were determined around the investigated disks. Experiments with each microbial strain were performed three times. The results were processed with the help of variation statistics methods.

Results

We have previously investigated the antimicrobial properties of medicinal films containing various common antiseptic agents in combination with weak acids (lactic, salicylic, succinic, orthophosphoric) [24]. The results proved films benefits after introducing decamethoxinum and chlorhexidine into biodegradable polymer-basis. At the same time we have proved that decasan in combination with lactic or salicylic acid increases antimicrobial films effect against putrefactive cocci flora (staphylococci, enterococci, β-hemolytic streptococci). This work is a logical continuation of research initiated and attempted to evaluate the biodegradable films antimicrobial effect made by our previously introduced method with adding zinc, magnesium nano-oxides particles, sized of 30 nm (Yurui (Shanghai) Chemical Co., Ltd., China) and decamethoxinum.

Made by us film containing zinc nano-oxide, is characterized by high antimicrobial effect against all microbial cultures used, except *Pseudomonas aeruginosa* (tab. 2). No significant differences in diameter of microorganisms inhibition zones have been determined around zinc nano-oxide films from methicillin-resistant and methicillin-susceptible staphylococci. Coagulase-negative staphylococci (*S. epidermidis and S. haemolyticus*) and β-hemolytic streptococcus *S. pyogenes* revealed higher sensitivity to zinc nano-oxide than strains of Staphylococcus aureus *S. aureus*. Films containing 5 and 10% of ZnO appeared to produce larger areas of growth delay against most strains in comparison to the films containing 1% of ZnO. In contrast to zinc nano-oxide, the presence of magnesium in films hasn't proved to have any antimicrobial effect.

For a more detailed analysis of the zinc concentration impact in nano-oxide polymer films on the growth of different types of microorganisms, the obtained results are presented in diagrams.

Regarding the strains of S. aureus, the greatest antimicrobial effect was observed in the films containing decamethoxinum. Zones of MSSA growth inhibition, being under the influence of zinc nano-oxide films, were increasing in proportion to the increase of zinc concentration. Optimal antimicrobial effect was produced by the film containing 5 and 10% of zinc nano-oxide. Zones of MSSA growth inhibition under the influence of magnesium nano-oxide film were two times smaller in comparison to those under the influence of ZnO containing film of a similar concentration. Zinc nano-oxide films retained high activity against MRSA. MgO film appeared to be absolutely ineffective against MRSA.

Similar patterns were found in the study of films samples against coagulase-negative staphylococci. The best effect against both *MSSE* and *MRSH* was produced by 5% ZnO containing film. MgO film did not appear to be effective against coagulase-negative staphylococci either *MSSE* or *MRSH*.

β-hemolytic S. pyogenes appeared to be the most sensitive of all ZnO films samples, particularly in concentrations of 1 and 5%. However, under the influence of MgO film, growth inhibition of *β-hemolytic streptococcus* was not observed.

Regarding antybiotic sensitive *E. coli*, the most active film appeared to be 5% ZnO. 10% MgO film produced almost half weaker antimicrobial effect. Diameters of inhibition zones of antibiotic-resistant citrobacter culture under the influence of ZnO film at concentrations of 1 and 5%, were 1,6 and 1,3 times bigger in comparison to antibiotic sensitive *E. coli*. MgO films and films containing decasan did not produce any antimicrobial effect against *ESβL + C. freundii*.

Regarding polyantibiotic resistant *Pseudomonas aeruginosa,* all investigated films proved to have only bacteriostatic effect. The effect produced by 5 and 10% ZnO films was equal to the one produced by the films containing decasan. ZnO films effectively inhibited the growth of Candida tropicalis which is resistant to classical culture antimycotics. This antifungal effect of 5 and 10% ZnO films was even greater than the effect produced by the films containing decasan.

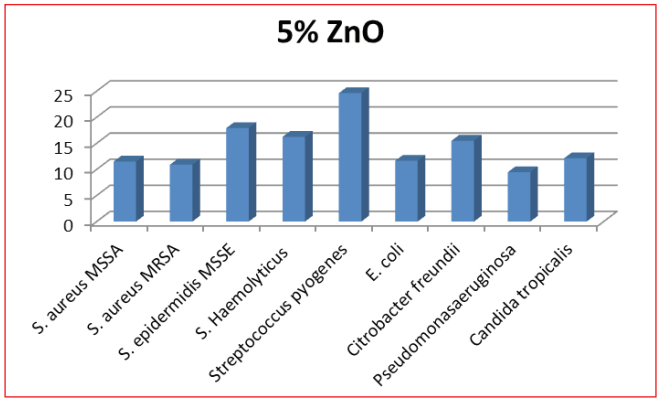

Fig. 1. Effect of 5 % ZnO films on the growth of microorganisms test strains.

The results of the experiments indicate that the optimum is to apply 5 % ZnO into biodegradable film basis. This type of films has the widest range of antimicrobial effect. They effectively inhibited the growth of all test strains, especially *S. pyogenes*, coagulase-negative staphylococci (including *MRSH* and S. *haemolyticus*), and the yeasts Candida tropicalis (fig.1).

Discussion

Antimicrobial properties of zinc oxide ions and its salts are well known and have been used in medicine for a long time [25]. In recent years, practical application of zinc oxide nano-particles has been intensively investigated [17, 20]. Into surgical practice there have been introduced and incorporated antimicrobial biomaterials as well as metal substrates [26]. There were applied fabrics impregnated with zinc nano-oxide [27]. Nano-particles of zinc oxide with gentamicin adsorbed have been reported to provide a synergistic effect of both components regarding *S. aureus*, *E. faecalis*, *E. coli*, *Salmonella sp*., *L. monocytogenes, P. aeruginosa* [28,29,30]. We have applied ZnO biodegradable polymer films for the treatment of septic and infected wounds.

The ZnO biodegradable polymer films suggested by the author are characterized by high antimicrobial effects against all used cultures of opportunistic microorganisms.

It should be noted that similar MgO films did not produce any antimicrobial effect. This result is consistent with literature data that point to a distinct correlation of antimicrobial effect of magnesium nano-oxide film to the size of its particles. Thus, 24-hour contact with the MgO nano-particles which are smaller than 10 nm, leads to intensive cell death of *S. aureus* spores of *B. subtilis*. However, MgO nano-particles of 50 nm have the ability to inhibit the growth of *E. coli* and *B. subtilis* partially [31].

Table 2

Antimicrobial effect of the investigated polimer films (dimeters of microorganisms inhibition zones, mm)

		S. aureus MSSA	*S. aureus* MRSA	*S. epidermidis* MSSE	*S. haemo-ly-ticus* MRSH	*Strepto-coccus pyogenes*	*E. coli*	*Pseudomona saeruginosa*	*Citrobacter freundii*	*Candida tropicalis*
1	1% ZnO	9.78±0.17	11.47±0.84	15.39±0.46	6.17±0.37	23.08±0.42	8.88±0.27	0	14.33±0.79	9.77±0.34
2	5% ZnO	11.49±0.35	10.90±0.46	17.90±0.77	16.23±0.32	24.60±0.11	11.64±0.16	[9.46±0.18]	15.48±0.23	12.13±0.89
3	10%ZnO	11.98±0.33	10.53±0.18	15.78±0.60	15.43±0.55	18.21±0.63	9.40±1.06	[9.83±0.20]	11.00±0.43	13.20±0.67
4	1% MgO	6.45±0.63	0	0	0	0	6.63±0.64	0	0	0
5	5% MgO	6.32±0.17	0	0	0	0	6.83±0.15	0	0	0
6	10% MgO	6.01±0.31	0	0	0	0	7.01±0.42	0	0	0
5	Decame-thoxinum	14.29±0.21	13.65±0.25	13.87±1.10	15.81±0.77	20.96±0.68	8.92±0.47	[10.20±0.36]	4.82±0.67	11.69±0.36
K	Control (basis)	0	0	0	0	0	0	0	0	0

Note: in brackets, zones of partial growth inhibition are presented (bacteriostatic effect).

Thus, in vitro experiments showed that biodegradable polymer-based films made of gelatin, polyvinyl alcohol, lactic acid and glycerin also containing 5% and 10% of zinc nano-oxide proved to have high antimicrobial effect against Gram-positive and Gram-negative opportunistic microorganisms that are most common causative agents of surgical wound infections. It is important from a practical point of view as this effect spreads to polyantibiotic resistant strains. So, developed by us nano-containing biodegradable polymers can be used as a means of drug delivery in the treatment of septic and infected wounds of various origins.

Conclusions

1. Biodegradable films containing zinc nano-oxide have high antimicrobial effect against opportunistic microorganisms – pathogens of wound infections, including their polyantibiotic resistant strains.

2. In vitro experiments showed that biodegradable polymer-based films containing 5% zinc nano-oxide proved to have high antimicrobial effect.

3. New polymer biodegradable films with nano-oxide content have optimal antimicrobial effect in vitro against relatively opportunistic microorganisms and require further experimental and clinical studies.

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Surveillance of antimicrobials use in Emergency Medicine Institute

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Abstract

Background: Antibiotics have had a profound impact on humanity's health, by improving our ability to prevent, cure and reduce the transmission of many infectious diseases. It is widely known, that the unnecessary or inappropriate use of antibiotics, occurs up to 50% of prescriptions only in the United States and Canada. Fortunately all negative impact on the human health can be roughly imagined.
Material and methods: For this study we used the data of a six-year (2009-2014) period in the Emergency Medicine Institute and their main subdivisions which show the consumption dynamics of antibacterials use in natural indexes.
Results: The total annual medium consumption of antimicrobials was registered as the following: ICD 1796.98 DDD/1000, SSOTD 566.12 DDD/1000 and EMI 584.05DDD/1000, with the parenteral to enteral forms share of respectively 94.67% to 5.33%, 85.62% to 14.38% and 83.52% to 16.48%. Five from nine main groups: beta-lactam antibacterials, penicilins, other beta-lactam, aminoglycoside, other antibacterials and quinolone antibacterials registered around 90% of all antibiotics consumption. Comparatively to Australian hospitals and hospitals other worldwide countries in EMI consumption per DDD/1000 was lower: by 3.39 and 2.22 times for tetracyclines, by 5.1 and 4.63 for beta-lactam and penicilins, as well as by 2.55 and 1.63 for macrolides and lincosamides.
Conclusions: The obtained data about the dynamics of antibacterials consumption in EMI and their main departments, in comparison with hospitals from other worldwide countries, represents important arguments and reserves for improving quality treatment, planning, rational prescription and utilization of antibiotics in hospitals.

Key words: antibacterials, defined daily dose, consumption, hospitals, utilization, occupied-bed days.

Introduction

Many surveillance drugs consumption programs, [1, 2, 3, 4] and strategies, [5, 6, 7] are used to achieve a prudent use of antibiotics in medical care institutions and quality of the anti-infective treatment of hospitalized patients. Nevertheless, a large proportion of antibiotic prescriptions is inappropriate, and constitutes up to 50% of prescriptions only in the United States and Canada. That's why, it is important to reduce the misuse and overuse of these important resources [8]. The primary aim of the study was to evaluate the institutional representative data on utilization of main antibacterial groups like tetracyclines, amphenicols, beta-lactam antibacterials and penicillins, other beta-lactam antibacterials, macrolides and lincosamides, aminoglycoside antibacterials, quinolone antibacterials, other antibacterials and antymicotics in accordance with the World Health Organization (WHO) requirements, which are directed to determine the value of Defined Daily Doses per 1000 Occupied-Bed Days (DDD/1000) [9] in the dynamics per total institution and most important departments, and to be compared with the same published data in international scientific journals.

Material and methods

For this study we used the data of a six-year (2010-2014) period consumption of antibacterials in Emergency Medicine Institute (EMI) and its main subdivisions: Intensive care departments (ICD), that include (Reanimation, Intensive Therapy and intensive "STROKE" departments) as well as SSOTD (Septic surgical and Septic Orhtotraumotology departments) which show the dynamics of consumption of main groups of antiinfectives for systemic use drugs as classified by Anatomical Therapeutic Chemical (ATC), classification system of the World Health Organization (WHO) indicated in grams and value indexes. Statistical, analytical, mathematical, comparative, logical and descriptive were used as the methods of study.

Results and discussion

For determining the number of DDD/1000 we used data about total annual consumption of antimicrobials and the statistics data concerning the number of treated patients (only patients with health insurance and other free treated by the state categories of citizens) [10].

Despite the fact that the use of tetracycline in hospitals holds some of the last positions in consumption among other antibiotics subgroups, antimicrobial therapy treatment of severe acute respiratory diseases (SARS) includes tetracycline (91.0%) and its combination with other antibiotics: tetracyclines and aminoglycosides in 18.8% of the patients, tetracyclines and quinolones in 11.5%, also 63.5% received a combination of tetracyclines, aminoglycosides and quinolones, [11, 12]. In some hospitals from other countries medium consumption for tetracycline recorded the following 0.5-27-70.00 DDD/1000 [13]. Tetracyclines consumption in EMI is characterised by the use of doxycyclinum with 0.1g defined daily doses. In the last 3 years of the evaluated period a total considerable increase of consumption was recorded: from 21.07 to 143.22 DDD/1000 or by 6.80 times in all departments, from 5.9 to 106.6 in intensive Therapeutical department and from 1.41 to 25.6 DDD/1000 in septic Surgical department.

During the last decades in many countries of the world, Amphenicol consumption was limited from 0.62 to 0.01% or totally absent [14, 15]. Notwithstanding, due to the increase of antimicrobial resistance to all antibiotics, in some countries has been renewed the interest to old drugs that have fallen into disuse. One of such examples is Israel, where Chloramphenicolum with susceptibility is routinely assessed in 44.4% – 83.3% of hospitals mostly for the treatment of aspiration pneumonia, [16]. In EMI the medium institutional amphenicols consumption constitued less than 1 DDD/1000 and recorded an increase during the years 2009-2014 from 0.5 to 0.8 DDD/1000 or by 40%, as well all in the evaluated departments of 5.37 to 9.45 DDD/1000 or by 175.98%.

The beta-lactam antibiotics remain the most heavily used antibacterials in clinical medicine. The annual consumption in US is estimated to be in the range of 10–30 million tons, which continues to increase, [17, 18]. From all antibiotics in hospitals, the use of beta-lactam recorded in mean 30-50%, [19, 20]. In EMI consumption of this group is characterised by use of parenteral (P) and enteral (E) forms of Ampicillinum with DDD 2.0 P and 2.0E, Amoxicillinum DDD 1.0 P and 1.0 E, Amoxicillinum+ Acidum clavulanicum ICD with DDD 3.0 P and 1.0 E, Ticarcillinum DDD with 15.0 P. Total beta-lactam antibacterials and penicillins consumption in main departments of EMI in DDD/1000 during 2009-2014 is shown in figure 1.

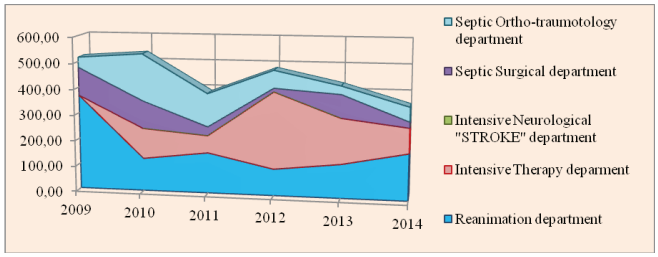

Fig. 1. Total beta-lactam antibacterials and penicillin consumption in DDD/1000 during 2009–2014.

From figure 1, it could be observed a total decrease of consumption of the group of antibiotics from 516.88 in 2009 to 356.82 DDD/1000 in 2014 or by 25.40%. From the annual medium consumption of 562.51 DDD/1000 could be placed as follows: the first – Reanimation department with 178.08 DDD/1000 or 31.66%, the second – Intensive Therapy department with 131.82 DDD/1000 or 23.43%, the third – "STROKE" intensive care department with 105.25 DDD/1000 or 18.71%, the fourth – septic Orhtotraumotology department with 84.37 DDD/1000 or 15.00% and septic Surgical department with 62.99 DDD/1000 or 11.20% on the fifth position.

All around the world in hospitals the consumption of other beta-lactam antibiotics recorded in mean 15-20% of all antibiotics, [21, 22, 23], whereas in EMI 50-60%. That situation determined a higher attention for this group of anti-infectives for systemic use. Total other beta-lactam antibacterials consumption in DDD/1000 during 2009-2014 is shown in figure 2.

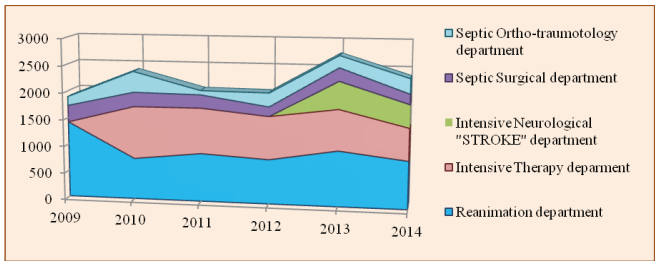

Fig. 2. Total other beta-lactam antibacterials consumption in DDD/1000 during 2009–2014.

From figure 2, it could be observed a total increase of other beta-lactam antibacterials consumption for all departments from 1893.69 DDD/1000 in 2009 to 2373.49 DDD/1000 or by 25.30%. According to the all departments annual medium consumption of 2701.58 DDD/1000 is placed as following: the first – Reanimation department with 970.38 DDD/1000 or 35.92% and a decrease from 1416.54 to 886.7 DDD/1000 or by 37.34%, the second – Intensive Therapy department with 794.95 DDD/1000 or 29.43% and a decrease from 974.67 in 2010 to 597.7 or by 38.68%, the third – Intensive Neurological "STROKE" department with 467.76 DDD/1000 or 17.31% and a decrease from 509.6 in 2013 to 425.95 or by 16.42%, the fourth – septic Surgical department with 237.92 DDD/1000 or 8.81% and a decrease from 310.05 to 187 DDD/1000 and septic Orhtotraumotology department with 230.57 DDD/1000 or 8.53% and an increase from 167.1 to 276.14 DDD/1000 or by 65.25% on the fifth position.

Consumption of macrolides and lincosamides antibacterials in EMI is characterised by use of parenteral (P) and enteral (E) forms as folowing: Erytromycin DDD 1.0 E, Midecamycinum DDD 1.0 E, Clarithromycinum DDD 0.5 EP, Azithromycinum DDD E0.3, P.5, Lincomycinum DDD 1.8 P.

A total decrease of macrolides and lincosamides consumption for all departments constituted from 108.77 in 2009 to 26.56 DDD/1000 in 2014 or by 75.58% and varied considerably in every subdivision during the evaluated period.

In hospitals from other countries, medium consumption for aminoglycosides recorded the following: 40.00 to 50.00DDD/1000 or not more than 5% of all amounts of antibiotics. At the same time consumption in ICD for critically affected patients is higher and varied between 38-66%, [22, 23]. In EMI, aminoglycoside antibacterials are presented by use of parenteral forms of the following antibiotics: Streptomycinum 1.0 P, Gentamycinum 0.2 P, Kanamycinum 1.0 P and Amikacinum 1.0 P defined daily doses.

In figure 3 the total consumption of aminoglicosides in DDD/1000 during 2010-2014 is shown.

Figure 5 demonstrates a total decrease in consumption of aminoglycosides for all departments during 2009 and 2010 from 780.30 to 677.89 DDD/1000 and a steep increase to 982.25 DDD/1000 in 2013, followed by a spontaneous

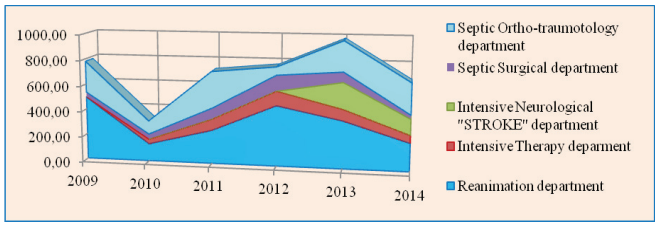

Fig. 3. Total consumption of aminoglycosides in DDD/1000 during 2010-2014.

decrease to 677.27 DDD/1000 in 2014 or by 31.05%. From the annual medium consumption of 832.24 DDD/1000, in all departments, the standings are the following: the first – Reanimation department with 326.37 DDD/1000 or 39.21%, the second – septic Orhtotraumotology department with 196.80 DDD/1000 or 23.64%, the third – intensive Neurological "STROKE" department with 165.38 DDD/1000 or 19.17%, the fourth – intensive Therapy department with 76.31 DDD/1000 or 9.17%, and septic Surgical department with 67.47 DDD/1000 or 8.11% on the fifth position.

Quinolone, is a broad-spectrum antibiotic, which plays an important role in the treatment of severe bacterial infections, especially hospital-acquired infections and has one more priority recommended as first-line therapy [24]. Consumption in EMI is characterised by the use of parenteral (P) and enteral (E) forms as the following: first-generation: Acidum pipemidicum ICD DDD 0.8 E,P, second-generation: Ofloxacinum DDD 0.4 E, Ciprofloxacinum DDD 1.0 E, 0.5 P, fourth-generation: Gatifloxacinum DDD 0.4 E,P and Mofloxacin with DDD 0.4 E,P. During the period 2009 to 2013 a total steep decrease from 583.42 to 185.63 DDD/1000 in consumption of quinolone antibacterials, followed by a significant increase to 469.65 DDD/1000 in 2014. In comparison with the annual medium consumption of 468.16 DDD/1000 the main three positions could be placed as the following: the first place – Reanimation department with 213.16 DDD/1000 or 45.62%, the second – intensive Neurological "STROKE" department with 84.04 DDD/1000 or 17.95% and the third – Intensive Therapy department with 80.26 DDD/1000 or 17.14%.

Consumption of other antimicrobials in EMI is characterised by the use of parenteral (P) and enteral (E) forms as the following: glycopeptide antibacterials: Vancomycinum DDD 2.0 P, imidazole derivatives: Metronidazolum DDD 1.5 P, nitrofuran derivatives: Furazidinum DDD 0.2 E, Nitrofurantoinum DDD 0.2 E and other antibacterials: Dioxydinum DDD 0.7 P and Nitroxolinum DDD 1.0 E.

Total other antibacterials consumption in DDD/1000 during 2009-2014 is shown in figure 4.

From figure 4, it can be stated a considerable decrease of other antibacterials consumption during 2009 and 2012 from 607.50 to 312.92 DDD/1000 of the total consumption or by 48.49%, followed by a steep increase to 551.08 DDD/1000 in 2014 or by 76.11%. According to the all departments annual medium consumption of 477.3 DDD/1000, could be placed as follows: the first – intensive Therapy department with 244.42 DDD/1000 or 51.22%, the second – Reanimation department with 167.7 DDD/1000 or 35.14%, the third – intensive Neurological «STROKE» department with 88.31 DDD/1000 or 18.5%, the fourth – septic Surgical department with 47,06 DDD/1000 or 9.86% and septic Orhtotraumotology department with 29.35 DDD/1000 or 6.15% on the fifth position.

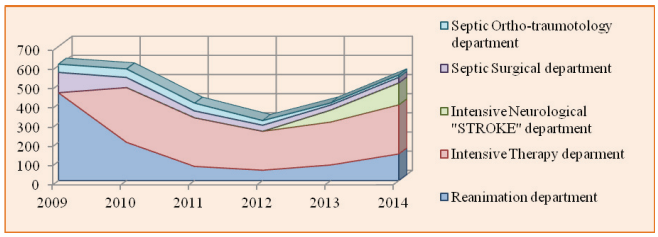

Fig. 4. Total other antibacterials consumption in DDD/1000 during 2009–2014.

Despite the fact that the use of antibiotics in many parts of the world has been described in details, the data concerning the use of antimycotics for systemic use such as imidazole derivatives and triazole derivatives are scarce, [25, 26]. Consumption in EMI is characterised by use of parenteral (P) and enteral (E) forms as the following: imidazole derivatives: Ketoconazolum DDD 0.2 E and Triazole derivatives: Fluconazolum with DDD 0.2 E, P. A total decrease in consumption of antimycotics for systemic use for all departments during 2009 and 2010 from 190.65 to 131.48 DDD/1000 and a steep increase to 231.22 DDD/1000 in 2013, followed by a spontaneous decrease to 108.91 DDD/1000 in 2014 or by 52.88%. More than 100 DDD/1000 from the annual medium consumption of 198.54 DDD/1000 in all departments recorded only Reanimation department with 105.25 DDD/1000 or 53.01%.

Evaluation of consumption of main groups of antimicrobials for systemic use shows a medium annual (for 6 years) period for: Reanimation department of 1981 DDD/1000, intensive Therapy department of 1192 DDD/1000, intensive Neurological "STROKE" department of 965 DDD/1000, septic Surgical department of 965 DDD/1000 and for septic Orhtotraumotology department of of 657 DDD/1000. A total decrease since 2009 until 2014 of antimicrobials consumption for ICD from 3273.79 to 1281.71 DDD/1000 or by 60.85%, for SSOTD from 631.14 to 515.54 DDD/1000 or by 18.32% and for EMI from 662.4 to 464.1 or by 29.94% during the evaluated period was recorded.

To determine the correlation between parenteral and enteral forms of evaluated antibiotics, was counted total by forms DDD/1000 separately for ICD and SSOTD and divided by the number of those departments (3 and respectively 2). The results are shown in table 1.

Data from table 1 shows the total annual medium consumption during 6 years, recorded for ICD 1801.00 DDD/1000, SSOTD 581.72 DDD/1000 and EMU 584.05 DDD/1000. Parenteral and enteral forms of use from total

Table 1

Correlation between parenteral and enteral forms of consumption of maim group of antibacterials in DDD/1000 in ICD, SSOT departments and EMI

Departments of and EMI	ICDD			SSOTD			EMI		
Groups of antibacterials/ forms of use	P	E	T	P	E	T	P	E	T
Tetracyclines	0	9.6	9.6	0	11	11	0	15.25	15.25
Amphenicols	3.52	0.27	3.79	0.63	0	0.63	0.65	0.25	0.9
Beta-lactam and penicilins	174.39	3.9	174.39	52.21	21.48	73.69	54.4	11.1	65.5
Other beta-lactam	895.3	0.48	895.78	221.8	12.42	234.22	249.89	12.38	262.27
Aminoglycozide	192.83	0	192.83	132.1		132.1	76.23	0	76.23
Macrolides and lincosamides	14.24	4.49	18.73	38.86	1.65	40.51	33.07	1	34.07
Quinolone	161.9	13.1	175	11.6	33.6	45.2	37.4	35.6	73
Other antibacterials	209.28	21.87	231.15	36.8	2.87	39.67	34.82	3.5	38.32
Antimycotics	49.71	46	95.71	0.11	15.6	0.11	1.31	17.2	18.51
Total	1701	95.81	1796.81	494.1	72.02	566.12	487.77	96.28	584.05

DDD/1000 consumption in ICD departamentals represent 94.46% to 5.54%, in SSOTD departments respectively 84.94% to 15.06%, as well as for EMI 83.52% to 16.48%. Five from nine main groups: beta-lactam antibacterials and penicilins, other beta-lactam, aminoglycozide, other antibacterials and quinolone antibacterials of the total medium consumption represents 1673.05 or 92.90% in ICD departments, 524.78 or 90.21% in SSOTD departments and respectively 515.32 or 88.23% in EMI. In table 2, comparative medium data of antimicrobials consumption per DDD/1000 in EMI and some international hospitals and perceptual change during 2009 to 2014 years are shown.

The results from table 2 show that during the evaluated period tetacycline consumption recorded an increament by more than 250% in EMI and Australian hospitals, when in the second case, it was recorded by 3.39 (51.75:15.25) as well as by 2.22 (33.80:15.25) times higher in other international hospitals, [27, 28, 29, 30]. The similar report by 5.1 and 4.63 times more could be mentioned for beta-lactam antibacterials, penicilins, as well as for macrolides and lincosamides by 2.55 and 1.63 times more. A decrease in consumption was recorded for aminoglycozide antibacterial, quinolone antibacterials and other antibacterials.The percentage changes in consumption of antimicrobial for systemic use for other international hospitals can't be counted, because evaluated data were for the period of 1 or not more than 2 years.

Conclusions

1. Five from nine main groups: the beta-lactam antibacterials, penicilins, the other beta-lactam, the aminoglycoside, the other antibacterials and the quinolone antibacterials registered around 90% of all consumption. Comparatively to Australian hospitals and other hospitals in foreign countries in EMI consumption per DDD/1000 was less by: 3.39 and 2.22 times for tetacyclines, 5.1 and 4.63 for beta-lactam and penicilins, and by 2.55 and 1.63 for macrolides and lincosamides.

2. Medium annual consumption in the evaluated period recorded the following results: in Reanimation department 1981 DDD/1000, intensive Therapy department 1192 DDD/1000, intensive Neurological "STROKE" department 965 DDD/1000, septic Surgical department 965 DDD/1000 and in Septic Orhtotraumotology department 657 DDD/1000.

3. Total annual medium consumption of antimicrobials recorded for ICD 1801.00 DDD/1000, SSOTD 581.72 DDD/1000 and for EMU 584.05 DDD/1000, with the parenteral and enteral forms share respectively 94.46% to 5.54%, 84.94% to 15.06% and 83.52% to 16.48%.

4. A total decrease during the evaluated period of antimicrobials consumption for ICD from 3273.79 to 1281.71 DDD/1000 or by 60.85%, for SSOTD from 631.14 to 515.54 DDD/1000 or by 18.32% and for EMI from 662.4 to 464.1 or by 29.94% was recorded.

5. Obtained data about consumption dynamics of antibacterials in EMI and its main departments in comparison with

hospitals from worldwide countries represents an important argument, which encourages to improve the quality of the treatment, planning, rational prescription and utilisation of antibiotics in hospitals.

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REVIEW ARTICLES

Infravesical urinary tract obstruction and transurethral endoscopic approach of treatment

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Abstract

Backqround: The results of scientifical progress in recent years have contributed to the development of transurethral endoscopic surgery, with the implementation of new methods, less invasive, for the treatment of the lower urinary tract diseases of different genesis. Laser surgery, transurethral electorezection, electrovaporization, bipolar surgery (plasmakinetic rezection and vaporization) and the combination of these methods have a number of avantages over traditional open interventions and contribute to a significant change in the treatment approach of most urological diseases, including those causing infravesical obstruction (IVO).

Conclusions: The implementation of personalized medicine with correct selecting and pathogenetic motivation of the methods of treatement in management of the IVO have been made in several directions: prophylactics, diagnosis and treatment. Medical and social importance, variety of clinic manifestations and evolution, evident alternation of quality of life, high cost of diagnostic, conservative management and of surgical treatment determine the statuts of infravesical obstruction as a current problem from scientific and practical point of view and motivates the need of a deep study of disorders manifested through IVO, minimizing complications and the rate of their relapse.

Key words: infravesical obstruction, stricture, hyperplasia.

Introduction

Infravesical obstruction (IVO) is a polyetiological and pathological condition caused by a number of urological diseases, which lead to an impaired urinary elimination from the bladder because of an obstruction in the bladder neck or urethral region.

According to statistical data, infravesical obstruction occurs in 50% of men aged 60 years and 90% of those who reached the age 85. Among men with infravesical urodynamic disorders, obstructive uropathy occurs in 20-35% of cases, and hydronephrosis, as a complication, – in 3.8% of cases [1]. In 23.9% of cases, infravesical obstruction in older men is conditioned by the development of prostate sclerosis, whereas benign prostate hyperplasia (BPH) associated with sclerotic changes is the commonest one [2]. Obstructive uropathy, caused by prostate disorder occurs in 5% of new cases of hemodialysis, in population aged over 65 years [3].

Any kind of micturition disorders may significantly reduce quality of life, develop psychological problems, which can affect family and employment relationships, and lead to social isolation. Besides the life-threatening conditions patients may experience, micturition disorders can lead to serious physical and moral sufferings, caused by deep psychological trauma, sexual conflict, the onset of neurosis and neurasthenia. From the psychological perspective, IVO is often associated with depressive disorders and is the most difficult pathology to diagnose. The long-lasting evolution, persistent dysuria and frequent recurrences may sometimes arise a feeling of hopelessness among patients regarding their treatment.

Infravesical obstruction is caused by urological diseases, which lead to the impairment of urinary evacuation, micturition difficulties, retention of urine and other types of dysuria. Infravesical obstruction is a characteristic symptom related to the following diseases like (fig. 1): adenoma of the prostate or benign prostatic hyperplasia, prostate cancer, bladder cancer (particularly located in the bladder neck), prostate and bladder neck sclerosis, urethral stricture and obliteration etc. [1,4]. The urodynamic resconstructure of urinary tract and the remodeling of regional renal and pelvic blood flow occur in infravesical obstruction [5, 6].

Subvesical obstruction, which causes the impairment of urinary evacuation, leads to urinary infection of both lower and upper tracts, and may commonly develop into cystitis and pyelonephritis. The obstacle in the urinary passage causes disturbance of micturition, which may eventually become worse viz. stranguria; pollakiuria; urinary incontinence (paradoxical ischuria) and the presence of residual urine, which is a polyetiological and pathological process, characterized by symptoms of lower urinary tract,which still remains a difficult problem for fundamental and practical medicine [7, 8].

Recently, there have been made a number of scientific researches in order to determine the causes of infravesical obstruction and optimize the best-corrected medical and surgical methods of treatment [9, 10].

The latest scientific clinical trials and technical progress have changed considerably the traditional urological treatment approach for many urinary diseases (fig. 1), which may result in infravesical obstruction i.e. benign prostatic hyperplasia, strictures and extended obliterations of the urethra, bladder neck and prostate sclerosis etc. [11].

Infravesical obstruction is most commonly met in old age and, in most cases, is caused by the development of a hyperplastic process in the prostate [12,13,14]. Benign prostatic hyperplasia is one of the most widespread polyetiological diseases in men of elderly and senile age, which occurs due to the proliferation of the transitional zone of the prostate, and paraurethral glands and lead to lower urinary tract obstruction [2, 15, 16, 17].

In this regard, according to the epidemiologic data, HBP was detected in 40% of men aged between 50 and 60 years, in 50% of men over 60 and in 90%–100% – after 80 years [2, 9, 18, 19]. It is considered that most men, over the age of 50, show certain symptoms caused by BPH [19], whereas the high life expectancy rates of aging men lead to an increased number of patients suffering from this disease [15]. Lately, some authors [20] mention the growth of BPH morbidity rates in population amongst most of countries.

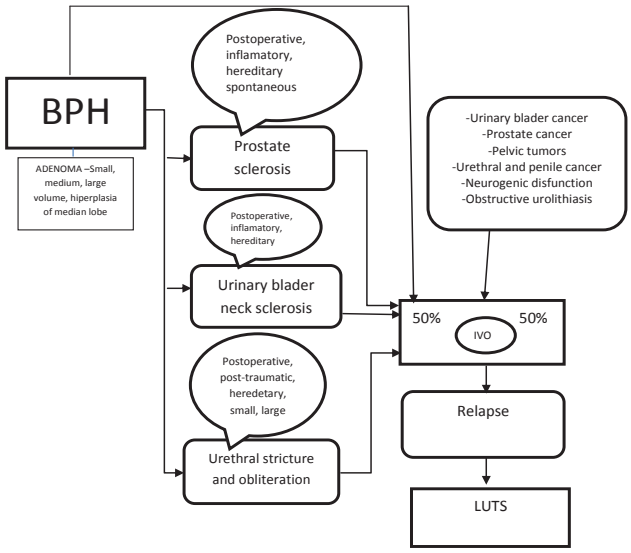

Fig 1. Urological disorders leading to infravesical obstruction. Infravesical obstruction caused by benign prostatic hyperplasia

The widespread BPH in men identifies the acute problem of timely diagnosis and well-reasoned treatment in the pathogenesis of this disease. It is extremely important, while choosing a reliable method of treatment for patients with BPH, to perform an accurate diagnosis of the disease and consider the shape of the prostate hyperplasia, degree of infravesical obstruction, prostate volume, as well [8, 21].

According to F. Schroder and I. Altwein [158], J.D. Mc Connell et al. [22], the clinical manifestations of BPH are detected in 34% of men aged 40-50 years, 67% of men aged 51-60 years, 77% of cases aged 61-70 years and 83% of men older than 70 years. Similar data were presented by other authors [23, 24].

In terms of clinical studies, the disease shows various symptoms related to the impairment of the urine passage through the lower urinary tract [25]. The causes of micturition disorder are infravesical urethral obstruction and underactive detrusor. The obstruction is caused by the enlargement of prostate with the gradual narrowing of the urethral lumen (mechanical component) and the hypertonus of smooth muscle fibres of the prostate and posterior urethra (dynamic component) (Fig. 2). On the basis of secondary changes of the detrusor with obstructed genesis, the stressors (direct influence of catecholamines) and ischemic (vasospasm) lesions of the smooth muscle elements of the bladder are of great importance. In these cases the bladder supports a large influence of catecholamines and, as a result of this process, disorders in bioenergetics and detrusor function occur [26, 27].

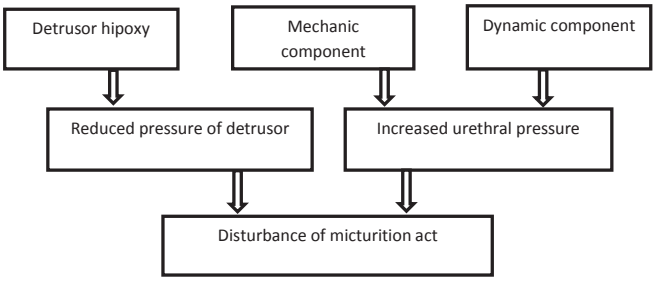

Fig. 2. The pathogenesis of impaired micturition in patients with BPH (A. Sivkov and co., 2009) [86].

Both theoretical and practical knowledge of the pathogenetic mechanisms present great importance in the development of obstructive uropathies. The research results of multilateral mechanisms of the pathogenesis of infravescal obstruction facilitate the detection of obstructive uropathies at the early stages, which enables the optimization of treatment methods in patients from the perspective of modern concepts, regarding the pathogenesis of this disorder, and allows the monitoring and timely performing of necessary changes in the selected treatment schemes.

Nowadays, the problem of benign prostatic hyperplasia treatment remains a current issue [17, 28, 29, 30]. Over the past 20 years, there has been an opportunity to treat patients at early stages of the disease using pathogenetically substantiated drug theraphies [8]. The successful obtained results, in the study of the pathogenesis of prostatic hyperplasia and the use of medicinal treatment with pathogenetic oriented mechanism, made the drug therapy possible and truly effective for certain categories of patients, affected by this disease. However, drug therapy does not relieve completely the patient's condition with prostatic hyperplasia, since pharmacotherapeutical methods show short-acting effectiveness and are applied to a limited number of patients with BPH (with Ist stage), therefore about 30-40% of men suffering from BPH, at present, remain subjects to surgical therapy [31, 32].

Unfavorable criteria characteristic for progression of prostatic hyperplasia are as follows:

– Prostate-specific antigen (PSA) is increased over 4 ng / ml in blood serum;
– Prostatic hyperplasia volume over 45 cm3 (the possibility of a prostate edema in acute inflammatory process should be considered);
– IPSS scoring more than 7;
– Low urinary maximum flow rate (less than 10 ml/s).

Additionally, the patient's age and associated diseases play a significant role in choosing the treatment techniques, especially in surgical treatment.

Modern urology provides many methods of treatment of this disease [9,15,33], but the most radical procedures are considered the surgical methods only, which include open adenomectomy and transurethral resection of BPH [31,34,35]. Modern Urological clinics perform TURP in 98.2% of cases and open interventions in 1.7-2.1% of cases [36].

In the treatment of large benign prostatic hyperplasia, transvesical adenomectomy has long been considered as main surgical method [28]. But despite its high efficiency, the rate of postoperative complications shows higher indices compared to endoscopic interventions. Thus, the total number of complications after a transvesical adenomectomy is between 12.5% – 23.02%, and according to C. G. Roehrborn data [37], this indicator reaches 38.5%. After transurethral resection, the postoperative complication rates range from 14.95% [22] to 18% [28,39]. Complications differ by structure as well; thus in late postoperative adenomectomy the incidence of bladder neck sclerosis ranges from 2.9% to 7.1% of cases [39,40], and stricture of the urethra – up to 5.1% [41]; while after transurethral resection, these parameters vary between 0.77% and 1.7% [25] and from 2.6% to 7, 14% of cases, respectively [28].

Therefore, a major issue of modern surgery today, is the choice of surgical methods of treatment for patients with late obstructive complications of transvesical adenomectomy [42]. Obstructive complications (bladder neck sclerosis and posterior urethral stricture), which are detected in 1.5% -9.2% of cases [43] may lead to the sudden worsening of the micturition and usually require extra special treatment, a correction performed by endoscopic therapy exclusively [38].

Transurethral prostatectomy or transurethral resection of the prostate is the second generation of surgical methods for the treatment of obstructive uropathies caused by benign prostatic hyperplasia

[44]. In fact, according to data of international meetings (American and European Association of Urology) for the treatment of prostatic hyperplasia, as well as different authors' opinions, transurethral resection of the prostate (TURP) is considered the "gold standard" treatment of BPH [29,33,38].

This method of treatment substituted open adenomectomy, and is applied in 70% -95% of patients as an alternative method to the surgical treatment procedures of BPH and infravesical obstruction caused by it [33,45,46] and also represents from 63.7 to 98.3% of all invasive procedures for treatment of prostatic hyperplasia [21,34]. Actually, this method is equally efficient compared to an open surgery and simultaneously, it shows a number of advantages like less trauma, lower risk of recurrence in patients, fewer complications, reduced hospital stays, shorter rehabilitation period, low postoperative mortality rate, etc. [15,17]. TURP implementation has contributed to considerable broadening of indications to surgical treatment for patients with BPH and pronounced recurrent diseases, who until recently were doomed to a lifelong elimination of urine through cystostomy [33,47,48].

However, it is worth mentioning, that in addition to its high efficiency, surgical treatment entails some risks of developing intra- and postoperative complications, which may occur in 8-22% of patients [9,12,49]. Additionally, in case of some categories of patients with BPH, open prostatectomy (adenomectomy) or TURP are life-threatening or contraindicated procedures, whereas drug therapy is inefficient due to the pronounced symptoms of infravesical obstruction [38]. Such patients are often bound to live with cystostomy, which seriously reduces their quality of life and leads to social inadequacy of the patient.

Despite its high clinical efficiency, TURP has a number of complications, which are detected in 25.67% of operated patients [9,17,50]. These include false and true recurrences of BPH, intra– and postoperative bleeding, TUR syndrome, urinary incontinence, bladder neck sclerosis, urethral stricture, retrograde ejaculation, etc.

Thus, according to A. Martov et al. [18] and N. Sergienko et al. [14], patients with BPH, following TURP presented early intraoperative and postoperative bleeding in 11.5% of patients, acute urethritis – 5.8%, acute epididymitis – 29%, acute urinary retention – 5.4%, maintainance of irritative symptoms in the late postoperative period – 10.6%, bladder neck sclerosis – 4.2%, urethral stricture – 6.9%, incontinence of urine – 1.6% of cases.

Complications of transurethral resection are divided into early and late intraoperative and postoperative ones [9,17,18]. During intraoperative and early postoperative periods, the following complications may occur: profuse hemorrhage, signs of water intoxication (TURP syndrome), urinary tracts injury, acute urinary retention, infections and inflammatory complications; whereas during late postoperative period , complications like maintainance of irritative symptoms, urethral stricture, bladder neck sclerosis, incontinence of urine, disease recurrence, retrograde ejaculation may commonly occur. Many of these complications require repeated surgery [9,25].

Retrospective analysis of TURP complications [51] showed that the occurrence of intraoperative and early postoperative complications is related to the level of training of TURP techniques (it has been reduced from 21 to 4.2%). In this regard, a considerable attention is paid to the peculiarities of form and morphological structure of BPH, since the inflammatory complications occurred on the already pre-existing diseases (chronic prostatitis and chronic

pyelonephritis). According to the authors, scarce results of endoscopic interventions characterized by persistent irritative symptoms were determined, in most cases by dynamic components, simultaneously with detrusor dysfunction. In this case, incorrect indications and unjustified surgical treatment play an important role in the development of complications and require further scientific and practical research.

The highest percentage of all postoperative complications refers to infravesical obstruction recurrence. According to M. Trapeznikova et al. [52], the recurrent postoperative IVO may be early (immediate) and late. In fact, early postoperative IVO recurrence has technical aspects of interventions, whereas late postoperative IVO is caused by scarring of the urethra and vesico-urethral portion (strictures and obliterations) and the continuous growth of adenomatous tissue (true or false recurrence of BPH) [9,25].

The existing scientific studies, regarding the problem of postoperative complications of transvesical adenomectomy and transurethral resection, as well as the measures used to prevent them, do not clarify a number of issues, such as: causes of complications in the postoperative period; their relation to the morphological structure of the prostate; the dependence of the late complications development on the early postoperative period development. Until nowadays, there is no clear picture of the recurrent causes of infravesical obstruction after BPH, mechanisms of its development, methods of treatment and prophylaxis.

According to A. Martov et al. analysis [17,18,53], the incidence of postoperative complications following the new endoscopic interventions of prostatic hyperplasia showed, that the major problem in the early period of standard TURP, is still a relatively high frequency of bleeding complications, especially in cases when blood transfusions were required. In the later periods of rotoresection and „vaporized" resection, there was a higher incidence of bladder neck sclerosis (10%) and urethral stricture after TURP in 6.9% cases. Therefore, minimally invasive alternative methods, such as TURP will successfully solve the problem of infravesical obstruction, whereas a number of complications restrict their use in clinical practice. According to the same group of authors [17], new transurethral endoscopic interventions highly reduce the number of intraoperative complications.

Despite the continuous improvement of surgical treatment, postoperative complications still remain at a high level of incidence and do not tend to decrease. There have not been sufficiently studied the particularities of clinical progression of large BPH [54], nor the development of it, as well as identification and treatment of infravesical obstruction in patients with median lobe hyperplasia of BPH.

Clinical manifestations of BPH are largely dependent upon the patterns of nodular hyperplasia growth and volume, which eventually lead to infravesical obstruction. It is a well-known fact that in patients with BPH, hyperplasia may occur in the lateral lobes, median lobe or all three lobes of the gland simultaneuosly [43].

There has been already established the role of so-called median lobe hyperplasia (MLH) of the prostate in the development of infravesical obstruction [55]. Some authors [56,57] consider the specific feature of median lobe of BPH, is inefficiency in treatment of this disease via different drug therapies.

Up to now, we have little information on the peculiarities of diagnosis and treatment of patients with BPH median lobe hyperplasia [58]. There are known only basic criteria that would contribute to an objective assessment of the participation degree of the median lobe hyperplasia, concurrently with the lateral lobes, in the evolution of infravesical obstruction in patients with BPH.

Complex research data on urodynamics (pressure-flow) also confirm the presence, characteristics and expressiveness of infravesical obstruction. It is also important to determine the role of an enlarged median lobe of BPH, which acts as a valve in the development of infravesical obstruction, and as a risk factor of possible complications following surgical interventions, as well as its effect on late treatment outcomes in such patients.

Traditionally, TURP treatment was applied to a relatively small prostate volume – up to 80 cm^3. But along with the improvement of transurethral surgery, this method has been used to remove the large prostate gland, as well, about 100-120 cm^3 (the mandatory requirement is sufficient experience of the surgeon). Larger BPH (over 80 cm^3) were detected in 10-20% of patients with this pathology. The removal of adenoma of such dimensions is possible through various means, including: transvesical and retropubic adenomectomy, transurethral resection, laser enucleation of the prostate, transurethral bipolar enucleation (TURBE). There have already been developed various techniques of transurethral interventions according to the size of gland hyperplasia. Thus, prostate vaporization is applied to a prostate gland volume of 30 cm^3, TURis BPH in 35-80 cm^3 volume, TURBE – 90-250 cm^3. According to A. Martov et al. (2014), transurethral enucleation in larger BPH (over 100 cm^3) is an alternative method to open adenomectomy or transurethral resection of the prostate (TURP). The method combines the minimally invasive TURP and radical pattern of open surgery. No doubt, minimally invasive interventions are more preferable compared to open adenomectomy. The removal of enucleated nodes can be performed via morcellators, by a common resection loop for TURP procedure and by cystotomic access for large bladder gallstones [54,59].Due to the obtained results following transurethral electroenucleation procedures using morcellators in HBP of various sizes, a number of authors were able to conclude [54,59] that transurethral electroenucleation of prostate (TURBE) by morcellation is an effective method to treat large BPH. The high speed of morcellator enables to reduce the time of intervention, and the obtained material is completely useful for histological research in terms of ensuring adequate irrigation and hemostasis quality.

However, TURP is not applied on a prostate gland volume of 150 cm^3 and larger, because of the high incidence

of intraoperative complications (bleeding, TUR syndrome, etc.), which will increase in number along with the hyperplasia tissue growth following an electroresection [12,19,49].

Moreover, complications after standard transurethral resection are more likely to occur i.e. the intraoperative and postoperative hemorrhage (in 0.9 to 10% of patients) and " water intoxication" syndrome (0.1-1% of patients) have led to the onset of minimally invasive alternative technologies, including endoscopic treatment of patients with HBP – methods that show efficiency and are comparable to TURP and would decrease the number of complications and failures [12,25,49].

The list of alternative technologies has expanded considerably in recent years and includes drug therapy, stenting, balloon dilatation, hyperthermia and thermotherapy, ultrasound and needle ablation, interstitial clotting, transurethral microwave therapy(TUMT) , as well as administration of ethyl alcohol (ethanol) and Botox, etc. Although, these techniques decrease the number of complications, they still cannot be compared to transurethral resection in terms of efficiency, both clinically and economically [60]. These methods have not been widely used either due to their inefficiency or because of the unfavorable complications [15,60].

The current, third period in the treatment of BPH is characterized by the rapid development of new endoscopic technological methods, which do compete with TURP in BPH treatment, being considered as the basic method of treatment during the last decades.

Bipolar transurethral resection of the prostate [30], Holmium laser (HoLEP) ablation (enucleation) [54,61,62,63,64,65] and transurethral vaporization of the prostate (TUVRP) show new means of BPH treatment [61,63,66].

Each of the methods has its own advantages and disadvantages. Availability of instruments, surgical skills and the indications for one or another method may largely determine the successful outcome. TURP of the prostate can be monopolar or bipolar; using the thin or thick loop [29,67]. Bipolar TUR of the prostate is associated with less bleeding and low risk of developing dilutional hyponatremia.

In the early 90s, the development of endoscopic electrosurgical technologies of prostate has led to the onset of a new method: Transurethral electrovaporization. Electrovaporization combines the advantages of the standard transurethral resection (immediate tissue removal, optical control of intervention, clinical and economical effectiveness) and a considerable decrease of intra– and postoperative bleeding ,as well as prevention "of water intoxication" of the body [42,49]. Transurethral vaporization, which uses thick cuneiform loop, causes vaporization and resection of the prostatic tissue, which is followed by a less hemorrhage and characterized by a shorter duration of intervention [42,68].

However, the practice shows that electrovaporization of prostate has its own disadvantages, too. Thus, e.g. "roller vaporization," which is the easiest technique to learn from the entire spectrum of electrosurgical methodologies used in treatment of BPH (simple technical management, good endoscopic visibility, no bleeding), has a lower capacity for removal (ablation) of hyperplasia tissue compared to monopolar transurethral resection. This is due to the fact that, firstly, roller vaporization may remove (evaporate) less tissue per time unit than in resection; and, secondly, the vaporization of the tissue is much more durable and lasting, since the carbonized layer that is formed slows the process down and a rehydration of tissue is required to resume the effect of vaporization. All of these require the application of roller vaporization in terms of a monotherapy for adenomas volume not exceeding 40 cm3 [69]. Vaporized resection, on the contrary, can be compared to standard transurethral resection in terms of efficiency and time of performance, but still it is as hard to be learned, because of the haemostatic effect of vaporizing loop,which is not as pronounced as the "roller vaportrode " whereas the technique performed on sections and the removal of adenomas requires considerable experience and training [31].

In order to overcome these deficiencies and reduce the number of complications mentioned above, as well as to improve the treatment outcomes of BPH and expand indications for surgical treatment in patients with somatic symptoms, a new direction in endoscopy has been required. Bipolar surgery, which has developed new technologies – resection and plasmakinetic vaporization (Plasma Kinetic TM Gyrus), uses saline solutions as irrigation fluid ; no electric current flow is needed through the patient's body due to the placement of 2 electrodes directly into the instrument, compared to standard monopolar resection, where the passive electrode is placed on the patient's lower limb [70,71,72].

It is worth mentioning, there has been little research reported on this issue, so far. The clinical effectiveness, indications and contraindications for this technique have not been fully described. There is a lack of knowledge regarding specific complications of this method. A high interest is paid to the comparative study of clinical characteriastics of postoperative period and distant treatment outcomes in patients after a standard monopolar transurethral resection with a loop electrode and plasmakinetic transurethral resection. These describe the actuality of the subject, both from a scientific and practical point of view, as well as the need for a comparative study of the effectiveness of this endoscopic technique.

The number of patients with BPH is increasing continuously, due to the growth of elderly population, development of diagnostic techniques, higher average life expectancy and men's need to improve their quality of life. In the surgical treatment of BPH, transurethral resection shows a percentage range from 63.7 to 98.3% of all invasive treatment techniques of prostatic hyperplasia. It took almost 50 years for TURP to replace open prostatectomy and become a predetermined intervention. However, complications like bleeding, retrograde ejaculation and TUR syndrome limit the use of this method in a number of patients [68]. Currently, transurethral resection of the prostate (TURP), which is considered the "gold standard" for treatment of urinary obstruction caused by benign prostatic hyperplasia, is entailing a

real threat. New minimally invasive endoscopic laser methods are being implemented in patients with IVO, caused by prostatic hyperplasia (BPH), specifically laser enucleation [10,62,73,74,75], which allows to reduce the number of complications and patient's hospital stay.

Laser technologies have been successfully applied in medicine worldwide for more than 35 years. In the last 25 years, laser treatment has been used in the most specific medical specialties, and presents an ideal combination of cutting and coagulation properties.

For the first time, neodymium laser was applied to the coagulation of prostatic tissue in 1979 by R. Bowering et al. Since 1990-1994, there appeared first international scientific works [10,62,63, 76,77], including Russian scientists [54,59,61], regarding the successful use of laser radiation and, particularly, the neodymium laser with a wavelength of 1064 nm in the treatment of patients suffering from BPH.

At present, both contact or non-contact laser methods are being used for coagulation and vaporization of hyperplasia tissue of the prostate in order to decrease its volume and provide a proper flow of urine from the bladder [78].

Holmium YAG laser treatment of the prostate has developed from simple ablation, resection to the modern technique of enucleation. Holmium laser ablation of the prostate (HoLAP) is a painstaking process because of slow tissue ablation rate and is indicated only for small glands. HoLRP involves a partial enucleation of each prostatic lobe, which is then divided into small pieces of the lobe, still attached to the capsule. Although HoLRP was faster than HoLAP, it still has a longer duration than TURP. The use of morcellator led to the onset of HoLEP technique – Holmium laser enucleation of the prostate [76].

Holmium YAG laser resection is usually applied in cases when the prostate volume does not exceed 60 cm^3; enucleation is performed in larger prostate. It is known that laser surgery of BPH has both advantages and disadvantages. The advantages of this method include lack of intraoperative and postoperative bleeding and "TUR syndrome", reduced invasive and traumatic features, and a very important fact, the possibility to be performed in patients with high surgical and anesthesiological risks. The disadvantages of laser method are the prolonged postoperative urine discharge, longer duration of intervention, the relatively non-radical character of treatment, the late onset of dezobstruction, considerable postoperative dysuria. Recently, some authors [54,79] have been studying perspectives of mutual combination of these laser methodologies in surgical treatment of patients with BPH. The laser surgery of BPH, like other less invasive methods of surgical treatment of this disease, ensures a successful surgical outcome by reducing life-threatening complications [62,80]. However, despite the successful results, many issues of laser surgery of BPH, still remain uncertain.

Lower urinary tract obstruction caused by sclerosis of the bladder neck and prostate

Infravesical obstruction caused by bladder neck sclerosis is the result of the scarring process of the connective tissue subjected to inflammation in the region of bladder neck and which partially affects the muscular wall; or a result of excessive electrocautery in that area during TURP procedure, as well as excessive suturing of the BPH lodge following a classic adenomectomy.

Bladder neck sclerosis according to N. Lopatkin and A. Pugaciov [81] affects 65% of patients, A. Liuliko and T. Kodiri [12] recorded 77.4% of cases, whereas G. Bakiev [82] stated 80.9%. In its turn secondary BNS is one of the complications of late postoperative period in patients with benign prostatic hyperplasia, which occurs in 0.4 to 24.8% of cases, thus secondary BNS remains a serious problem in modern urology. In the study conducted by Ying-Huei Lee et al. [83] on a lot of 1.135 patients who underwent standard transurethral resection (TURP), 9.7% of patients developed BNS during the mean postoperative follow-up of 37 months. Small prostates were initially diagnosed in most of these cases [83].Another study conducted by W. Al-Singary et al. [84] on a lot of 900 patients during a follow-up of 4 years after mopolar TURP procedure, showed that 3.4% of patients developed BNS with a mean resected prostatic tissue weight of 11 ± 3.7 g. One of the causes of BNS development after the surgical treatment for BPH is the concomitant chronic prostatitis [15], whereas the onset of the sclerotic process starts in the postoperative period, following a trauma or acute inflammatory process in the bladder neck region [11]. Sclerotic processes most actively evolve in conditions of hypoxia, whilst inflammatory process will only worsen the situation [70,85].

The pathological condition may develop through the stricture or complete obliteration of the bladder neck and is characterized by IVO progression to full retention of the micturition, which further will require bladder drainage (cystostomy). In the latter case, the condition is associated with social inadequacy of the patient, development of chronic pyelonephritis, chronic cystitis and eventually bladder sclerosis.

The incidence of developing bladder sclerosis is different, according to the types of surgical interventions. Thus, there were recorded 1.7% to 3.9% of cases following transvesical adenomectomy, 2-10% of patients after TURP, 1.28% of patients treated with bipolar plasmakinetic resection, 0.5% to 3.8% of cases occurred after Holmium laser TURP. According to EAU Guidelines, the risk of developing this pathology is 4% in patients treated by TURP, 1.8% after an open surgery for BPH and 0.5% to 14.6% after radical prostatectomy for prostate cancer [86,87,88].

Therefore, secondary bladder neck sclerosis is one of the most common chronic complications following a surgical treatment of the prostate.

According to N. Nashivocnikova, [29,89], the incidence of bladder neck sclerosis after a surgical treatment of BPH is 15.5% and does not depend upon the surgical method, prostate size and patient's age, but it depends on the recurrence of hemodynamic disorders in the pelvis and the presence of inflammation in the prostate.

The presence of microcirculatory disorders in the blad-

der neck in patients with BPH is a pathogenetic factor in the development of basic sclerosis. It is detected in patients at already preoperative stage, lasts throughout the postoperative period and as a result of a chronic inflammation may lead to pathological scarring [85]. The improvement of microcirculatory parameters in bladder neck region in patients with BPH, prior to surgery and during the immediate postoperative periods, can prevent the developing of bladder neck sclerosis, whilst in patients with BNS help reduce the reccurrence rate from 7.9% to 0.82 % [70,85].

The severity of BNS and other chronic disorders of urodynamics of the lower urinary tract is determined not only by changes in LUT, but also by the urodynamics of upper urinary tract (UUT). UUT changes are often decisive in assessing the severity of the disease and the choice for surgical indications [90]. For this reason the study of disease severity in selecting indications for surgery is an important step in the treatment of bladder neck sclerosis [91].

According to bibliographical data, the diagnosis and treatment tactics of bladder neck sclerosis entail some difficulties, since many tests that determine the type of disorder, also may refer to other causes of infravesical obstruction, as well. Diagnosis is particularly difficult in the early stages, when there is no clear image of the degree of clinical characteristics and radiological data [4;50].

Detection of bladder neck sclerosis is based on patient's complaints regarding difficulties in urination or inability to empty the bladder in a natural way; on the information about previous surgeries and complicated evolution of immediate postoperative period. The disease is diagnosed via radiological examination and endoscopic methods.

Therefore, in BNS , the findings of ascending urethrograms may determine the free permeability of the urethra up till the bladder neck; whilst in the urethra stricture, it is detected in the distal region of the urethra (in relation to the bladder neck). In case of "Prevesical space", there is an extra cavity between the bladder neck stenosis and the constricted portion of the urethra, which is shown on the urethrogram. In order to determine the degree of manifestation and location of IVO, the ascending urethrography of contrast is performed; and if micturition is still present, then – uroflowmetry and ureteroscopy. The differential diagnosis of patients with other obstructive complications after previous surgeries is carried out in: urethral stricture, false urethral channels, "Prevesical space" and sclerosis of the prostate. Common symptoms for these conditions are difficulties in urination or its complete retention.

An important issue in contemporary urology is the treatment of bladder neck sclerosis, since it is being widespread, especially in men of older and senile age [92]. Postoperative bladder neck sclerosis is a complication of TURP or radical prostatectomy, which still remains an unsolved problem [93,94]; although, a number of surgical treatment methods are known for both primary and secondary BNS.

The purpose of treatment of bladder neck sclerosis is to restore the permeability of vesicourethral segment through various methods of interventions. There were submitted various proposals to solve this problem. Therapeutic options range from regular dilation with balloon with about 50% of recurrencies, to cold loop resection, TURP of bladder neck in order to place the stent and others. The indication for intervention is the sign of IVO.

The choice of the surgical type is largely determined by the degree of the bladder and kidneys disorder [86,90,95].

Until recently, the most of old aged and senile patients have undergone open resection for bladder neck sclerosis. This surgery, despite its effectiveness, was quite traumatic and led to development of complications [92].

Transurethral resection of scar tissue (TURP of bladder neck), originally described by Sachse in 1974 remains the standard method of treatment for bladder neck obstruction in men with postoperative bladder neck sclerosis [92], which compared to the open surgical intervention is described as being highly effective in treatment of infravesical obstruction and symptoms caused by it. It is considered less traumatic, gives chances for being repeated without any significantly increased risk for the patients and it obviously provides a shorter rehabilitation period etc. However, the proposed surgical treatment methods are complex, multilevel and often ineffective. The existing treatment methods can cause hemorrhage, hematomas and scar formation with subsequent recurrent relapses and may require repeated interventions. The above listed surgical interventions are considered inefficient since there is no assessment of the manifestation degree of the scarring process on the bladder wall, of the conditions of trigonal-urethral and vesicourethral regions, in terms of infravesical obstruction treatment.

There are several poorly highlighted aspects. These concerns include: a complex of necessary diagnostic methods, more accurately assessed indications and contraindications for transurethral and "open" resection for the bladder neck in patients with BNS, technical peculiarities of endoscopic and open surgeries to patients of this category, the study of intraoperative and postoperative complications of transurethral and transvesical resection of the bladder neck and their preventive measures, peculiarities of pre– and postoperative management of elderly and senile patients.

Since, the surgical intervention is considered the basic method in the treatment of BPH and complications are inevitable, the number of postoperative patients with prostate and bladder neck sclerosis, obviously increases [92]. Thus, M. J. Bader et al. assessed the obstructive complications rate from 1.1% to 24.8% of cases, after an adenomectomy; whilst according to T. Bach et al. [93], bladder neck sclerosis is observed in 3.6 to 17.9% of patients after radical prostatectomy or TURP.

Therefore, a further research and development of less traumatic alternative treatment options are required to be learned and implemented, as well as the improvement of already existing methods such as bipolar plasma vaporization [86,94], using various laser treatments with good results, including neodymium, argon and holmium (YAG laser) [64,93,96].

Prostate sclerosis is a disease, where the sclerosis parenchyma of the gland constricts the prostatic urethra, narrows the bladder neck and vesical sections of ureters, compresses the deferens channels, causes dysfunction during micturition and urinary stasis, causes a decrease in kidney function and disruption of various phases of the copulative cycle. Prostate sclerosis does not refer to the category of widely spread urological diseases. It raised the interest among urologists, since the infravesical obstruction caused by the prostate sclerosis is found in 52.8% of younger men (up to 59 years) and if left untreated ends up with the end-stage kidney failure. Comparative studies of the prostate sclerosis and BPH incidence have established a ratio of 1: 5 of these conditions in 1970-80 and 1: 3.2, in 1986-1995, which shows an increasing tendency of cases. Particularities of the prostate sclerosis process have been established. In younger men (up to 50 years) prostate sclerosis is often associated with focal hyperplasia of parenchyma and develops on the background of chronic inflammation. In patients older than 50 years, modified atrophic parenchyma, with cystic changes are detected [97].

According to the unanimous opinion of scientists, the prostate sclerosis is genetically related to prostatitis [98]. The inflammatory process in the gland is found in 62% of cases. Chronic Prostatitis leads to BPH in 40-100% of cases [99].

Prostate sclerosis (73%) is one of the end- stages of the chronic inflammatory process in the prostate (chronic prostatitis) [92,99]. This stage is characterized, as a rule, by a decrease in the inflammatory process activity; simultaneously, the organ is considerably replaced by a scar connective tissue [92]. Additionally, prostate sclerosis is a complication of the late period in patients who underwent adenomectomy (6%) or TURP (21%) related to BPH. In some patients, prostate sclerosis causes infravesical obstruction. It was determined that in 23.9% of elderly men, infravesical obstruction is conditioned by the development of prostate sclerosis and, that small BPH are most commonly associated with sclerotic changes in the prostate [92].

The treatment of prostate sclerosis is an important issue of the modern Urology regarding the widespread nature of this disease, especially in men of older and senile age [92]. Simultaneously, the increase in average life expectancy considerably raises the number of patients of elderly and senile age, who also present intercurrent conditions, thus increasing the risk for surgical interventions [40]. Intercurrent conditions of other organs and systems complicate evolution and basic treatment in 35.8% of patients. Since high-risk surgical patients are reported in 13% to 17% of cases, they do not undergo a radical surgery; cystostomy is performed instead, which leads to social inadequacy of the patient. Since the surgical intervention is considered the major method of treatment of BPH and the presence of obstructive complications is inevitable, the number of patients with prostate sclerosis increases, as well. Obstructive complications after adenomectomy are reported by V. Bazaev and A. Morozov [100] in 1.1% to 24.8% of cases. Thus, according to bibliographic sources, prostate sclerosis and benign prostatic hyperplasia are mostly prevalent among male urological in-patients, and therefore the effectiveness of treatment of these diseases is of great economic and social importance [2]. Currently, TURP is considered the most effective method of treatment of prostate sclerosis in order to remove the infravesical obstruction, [101; 102].

Analysis of surgical outcomes based on a lot of 165 prostate sclerosis patients and further improvement of urodynamic parameters, allowed L. Gorilovskii and M. Dobrohotov [92] to conclude that transurethral resection is an effective method to treat infravesical obstruction and restore the micturition , especially in patients of older age and senile, while improving the quality of life, as well. Beneficial results, regarding the improvement of symptoms after the surgical treatment of prostate sclerosis, were determined in patients with obstructive symptoms of the disease rather than irritative ones. From the morphological point of view it has been proved that the prostate sclerosis is a result of the so-called stromal hyperplasia, being one of the types of prostate hyperplasia. [92]. It is based on the proliferation of stromal elements, although in some cases lax muscle fibre type stromae forms a nodule of muscle fibre- type, so-called spheroids. The prostate in this case is rather consistent and slow-growing.

The main task in the surgical treatment of prostate sclerosis is to reduce the number of intraoperative complications, early and late postoperative complications and morbidity rate. In order to achieve this goal, it is necessary to reduce the number of open surgeries and increase interest for endoscopic treatment methods, specifically TURP, laser, and plasmakinetics.

However, despite the good results and the efficiency of TURP, it still does not lack complications like bleeding during and after surgery (arising in 0.9 to 10% of patients), as well as the body water intoxication syndrome – "TUR syndrome" (0.1 – 1% of patients), which sometimes lead to unfavorable results.

The mortality rate, even if it is lower than for open surgery, it still remains quite high, which is particularly important in elderly and senile patients [2]. All these aspects facilitate the search for optimized treatment technologies [92].

Subvesical obstruction caused by stricture and obliteration of the urethra

Urethral stricture is one of the most complicated urological diseases. The incidence of the stricture of the urogenital system diseases constitutes about 6% of cases [12]. It is actually thought, that the incidence of urethral strictures is much higher, since quite often patients, who complain of disorders in urination, are incorrectly diagnosed (i.e. atony of bladder, prostate adenoma, chronic prostatitis, chronic cystitis etc.). But the real disease, namely urethral stricture, is detected only during a further deep investigation, whereas in a number of cases, it remains undiagnosed [103].

Urethral stricture is the second most common cause of obstructive micturition disorders in men regarding the pros-

tate diseases [104,105]. In the last two decades, the incidence of urethral strictures significantly increased. This is due to several factors: on the one hand, the increased number of severe damages of pelvic organs [106,107]; and on the other hand, as a result of the largely implemented endoscopic methods and transurethral resection [107]. The incidence of posterior urethral stricture and bladder neck sclerosis is particularly high [23,105,108].

Until the widespread practical implementation in urology of endoscopic treatment methods, mostly, transurethral resection of the prostate in patients with urethral stricture, the major part was represented by patients who suffered a trauma. In this case, 47.7% of the patients were aged between 21 to 40 years. Traumatic stricture was found in 84.7% of patients, including 9.6% of cases, caused by adenomectomy and medical manipulations [31]. Over 10 years, the last figure rose to 14.7% [109]. The iatrogenic cause of stricture occurrence is found in 24% of cases, whereas adenomectomy and TURP were the causes of stricture development in 13.6% and 10.5% of cases, respectively. A slightly different situation is noticed in case analysis of urethral obliteration. In 40.7% of cases, obliteration is caused by traumas; 39.53% of cases showed an iatrogenic cause (transvesical adenomectomy – 33.72%, TUR of the prostate – 5.81%); obliteration arising after a previous plastic interventions in the urethra – 19.77% [89,110]. Patients with posterior urethral and bladder neck obliteration are the most difficult category of patients with urethral obstruction [82].

Urethral stricture is a condition characterized by various symptoms of lower urinary tract that are and depend on the cause, location and severity of the strictures [23]. The symptoms of urethral strictures (the urinary stream is characterized by a weak, thin, forceless, sometimes "dripping" flow) and possible complications (incomplete or complete retention of urine, fully distention of urinary tract and, even renal failure) may lead to confusion in the diagnosis of obstructive urinary diseases. Basic causes are commonly benign (infectious, ischemic, iatrogenic, traumatic, congenital, undetermined) or rarely, neoplastic (not examined in this study). The etiology of scar is characterized by traumatic, inflammatory and congenital causes. According to their location, they can be prostatic, membranous, bulbar, penile; according to their size (expansion) short (≤ 2 cm), long (≥ 2 cm); with subtotal damage in 75-90% of the spongy urethra, total spongy (total damage of spongy urethra) and total (total damage of the urethra); by number – single and multiple; according to the degree of narrowing of the urethra: mild – lumen is narrowed up to 50%; moderate – lumen is narrowed to 75%; severe – lumen is narrowed more than 75%; obliteration – no lumen.

Male urethral stricture is an obstructive polyetiologic disease, which involves urethral epithelium, spongy body, and in some cases paraurethral tissues. Due to the changes of scar tissue in the urethral walls, the progressive narrowing of the lumen diameter (in one or more places) occurs.

In elderly and senile men, a previous surgery is often the cause of urethral stricture or obliteration [82,105,106]. The list of interventions which lead to urethral strictures, most often includes the surgeries associated with benign prostatic hyperplasia: transvesical adenomectomy, transurethral resection of the prostate.

In recent years, it is complemented by radical prostatectomy performed in prostate cancer. Urethral stricture following such type of surgery, obviously, occurs in patients of advanced or senile age [16,23]. There is a high risk for a considerable number of patients to undergo urologic surgery, which is determined by a number of factors: increasing age of patients who undergo surgical treatment, along with elderly (61-74 years) and senile (75-90 years), often applied to long-lived patients (90 years and over); most patients with associated diseases are often subjected to surgical treatment i.e. lung diseases, chronic hypertensive disease, ischemic disease of the heart, anterior myocardial infarction with development of cardiosclerosis, disease of peripheral vessels, blood-vascular failure, consequences of impaired cerebral circulation, metabolic disorders such as diabetes, obesity, hormonal deficiency, drug intolerance [111].

Urethral trauma is ranked on the first place among the most male urogenital injuries [23,105,106]. One of the complications of severe urethral trauma, which leads to prolonged loss in work capacity and often to disability, is its narrowed scarring.

The increasing number of traumatic disorders of the urethra leads to the steady growth of number of patients with stricture scarring, in most countries [112,113]. Meanwhile, there is an increasing number of recurrent strictures, which proves the low efficiency of surgical treatment methods [23, 106,114,113,115]. Native authors [116,117,118] investigated the efficacy of treatment methods in open urethral strictures, especially the posttraumatic [116,118] and analyzed the outcomes after an endourologic treatment of acquired urethral stricture, specifically iatrogenic and post inflammatory urethral stricture, the peculiarities of urodynamic exam findings in patients with primary and recurrent acquired urethral strictures [118].

Even if urethral strictures have a relatively small share in urological pathology (3.7 to 6%), the issue of their treatment refers to the most current situations in modern urology [119]. This is due to the large number of complications and high recurrence rate (10-50%), which often lead to social inadequacy of this category of patients [120]. One of the current problems of urology is the treatment of urethral strictures of different genesis, including postoperative strictures with different locations, following an adenomectomy [23,109].

The successful interventions in the urethra depend on the choice of optimal surgical method while taking into account the location, length and complication of stricture; the appropriate use of intervention techniques; the postoperative treatment of patient; the overall condition of the body; the presence of recurrent diseases and complications [23,114,115,120].

Patients with urethral strictures require a specialized and prolonged treatment. Surgery is a radical treatment method performed by means of open or endoscopic technique. The choice of a surgical method is based upon specific indications and contraindications, some of which are still considered controversial and largely discussed in related literature. As for example, the choice for optical uretrotomy in extended urethral strictures and its complete obliteration [23,34].

Until recently, the main methods of treatment of patients with urethral strictures were open and complex surgeries (i.e.urethroplasty, urethral reconstruction) as Holtsov-Marion, Solovov, Rusakov, Mikhailovsky etc. [23] most of which does not guarantee a full recovery. Unsatisfactory results of treatment in patients with urethral strictures are commonly met and reach up to 16-25% of cases. The open surgeries also include: different methods of anastomosis, urethral plastic surgery, displacement of grafts [23]. The efficiency rate of listed above treatment methods constitutes about 80-95% and are applied mainly in extended urethral strictures [25], and the overall rate of recurrence is about 5-20% [102,106]. Besides its traumatic and long rehabilitation period, the most common complications following open surgery are: postoperative suppuration, incidence of urinary fistulas, stricture recurrence, urethral obliteration, urinary incontinence. The following side effects of surgery of the urethra should be reported: shortage of penis, erectile dysfunction and impotence associated with inevitable trauma of muscles, vessels and nerve endings of the perineum during the intervention as well as urethral resection [82]. All this leads to social inadequacy or disability of this category of patients.

Recently, great importance is paid to the development and implementation of modern technologies, especially minimally invasive methods of treatment of urological diseases. Various endoscopic surgical methods are widely applied, which enable the efficient restoring of urethral permeability in the early follow-up period; endoscopic surgery is performed in urethral obliteration [89,121]. Currently, transurethral methods are widely used in treatment of this disorder, which are divided into cold knife urethrotomy, electroresection and laser urethrotomy. These include mainly internal visual urethrotomy; visual dilation of the stricture and insertion of the endoscope during transurethral intervention of lower urinary tract; Otis urethrotomy, transurethral resection of the urethral sclerotic scar tissue [89,122].

Endoscopic treatment methods include internal optical urethrotomy; urethral endoscopic disobliteration; combined endoscopic interventions, which present the following advantages: minimum operational trauma; minimal duration of intervention; no significant intra– and postoperative complications. A number of authors have extensive experience of endoscopic treatment of urethral strictures, which allowed them to avoid complex open procedures, in some cases [89,122].

A palliative method to restore the micturition is internal visual urethrotomy – a minimally invasive endoscopic method of treatment, which actually consists of an incision performed on the narrowed portion of the urethra and stroma lumen by means of special instruments [120,123]; thereby a normal urethral lumen is obtained. This surgical technique is performed via a visual management, where intraurethral longitudinal incisions are made on the stenotic segment using an optical urethrotome [88,124]. The success rate of this surgical procedure is 30 – 40% within a year, whilst failures are considered secondary effects of this apparently simple endoscopic technique. The main purpose of this method is the cure, viz. obtaining a steady functional urethral lumen without a need for further investigation, therefore cases of strictures less than 1 cm will be selected, with moderate spongiosal– fibrous process, located at level of bulbar urethra.

Visual urethrotomy enables to perform a selective and precise incision, which prevents the emergence of many complications. The disadvantages of this method are: the prolonged follow-up period of patients, whereas its success depends on the intellectual level and readiness of the patient [125,103,126].

In order to improve a relatively modest success rate (up to 30-40%) on a long– term period, urethral stents (prostheses) can also be placed endoscopically at the level of stenotic urethral segment aiming to maintain the proper function of the urethral lumen. Except for its high price, this method does not decrease the recurrence rate of stenosis, and it is extremely difficult to treat a prosthetic and recurrent stricture.

During recent years, there have appeared a series of scientific works regarding the benefits of this method (visual internal urethrotomy) [25,110,126,127] in the treatment of post-traumatic urethral strictures [128] and postinflamatory ones [119,128]; as about the treatment of postoperative urethral strictures the results are more diverse and specific [129], according to reported data. Perhaps this is due to some characteristics of stricture location following an adenomectomy, as a rule, placed in the proximal segment of the urethra and on the bladder neck region, or after a urethroplasty [130], as well as to the difficulties of surgical access,a fact which complicates considerably the choice for surgical technique. Finally, the main idea of all these communications was that visual internal urethrotomy is an effective method of treatment of urethral stricture, which is characterized by less traumatic and better results. IVO is the surgical approach selected in less expanded urethral strictures; it may prevent the patient from undergoing repeated open plastic surgeries or reduce post urethroplasty recurrence [126,131]. If associated with appropriate anti-scar treatment during the postoperative period, it may significantly reduce the number of recurrence and improve treatment outcomes [128,132,134].

In specialized literature, there is no unanimous opinion regarding the indications for IVO. M. Trapeznikova et al. [52,136] thinks that this method can be applied to all strictures, regardless of their etiology, level of manifestation or impairment. The purpose of these procedures is the formation of the tunnel, as well as localization of a preserved portion of the urethra. Some authors [134], made attempts to

use retrograde visual urethrotomy in treatment of urethral obliteration, but due to high trauma rate and probability of false paths onset (false urethra) ,this method has not been widely applied in practice. Surgical intervention is indicated only in cases of short urethral deficiency, the bladder neck is competent and there is a minimum displacement of the prostate and bulbar proximal urethra [119]. Although, the restoring of continuity is somewhat simple and potency is not affected by the procedure, still urethral dilatation, repeated visual urethrotomy and transurethral resection of stricture are very commonly used in 82% of patients. Most repeated urethrotomies are made in the first– year of follow-up. It should be noted that in case if initial urethrotomy fails, alternative methods of treatment are applied, since repeated urethrotomy leads to only temporary improvement. Besides a false urethral path, a rectal perforation may often occur.

According to bibliographic data, recurrences following IVO occur in about 80% of cases; after Frank M. [41], V. Bazaev, S. Urenkov [82] – in 15-50%, and according to A.M. Naude [124] Giannakopoulos, et al. [135] – in 50% of patients. A number of authors [126; 136] reported about the high incidence of remote recurrence in treatment of patients with urethral stricture of inflammatory etiology by means of endoscopic urethrotomy.

Some authors [119] convincingly demonstrate that visual internal urethrotomy is an operation of urethral stricture selection and the treatment of each patient must be initiated with visual urethrotomy. VIU is now a simple, accessible and technically easy to perform procedure. But this intervention can not be considered pathogenically argumented. Urethrotomy is a palliative surgery [122]. Any induced and unstiched wound, heals by scarring by secundam and formation of scars, therefore stricture recurrence is inevitable.

Therefore it is not surprising that the supporters of visual internal urethrotomy recommend the mandatory long-time survey, sometimes for life, while admitting however multiple repeated urethrotomies. According to several authors [122], the strictures recurrence in visual internal urethrotomy ranges from 30.8 to 58.2%. D. Pushkari et al. [120,137] reported stricture recurrence after internal visual urethrotomy in 20-80% of patients.

Additionally, cold knife urethrotomy and diathermocoagulation are used in stricture incision along with laser irradiation has been positively assessed by several authors [72,138]. In opinion of I. Karpuhin et al. [119]; A. Martov et al. 103; 110] V. Voinescu et al. [139]; C. Moldoveanu [86]; M. Lucan et al. [140], urethral strictures laser therapy is an alternative approach to classical cold knife urethrotomy. The best results are obtained in non-recurrent strictures of less than 1 cm long. In cases of recurrent strictures longer than 1 cm long, the alternative to open surgery should be considered. In severe cases with multiple recurrences, the method does not provide satisfactory results, whereas urethroplasty is considered the best indication for these patients [139,141].

Thus, according to some authors [58,103,142], the use of the holmium laser in the treatment of extended urethral strictures showed good results in 80.9% of cases, whilst the recurrence rate of the disease being of 5.5%; while in the treatment of complete urethral obliteration following repeated surgical interventions, this method of treatment may be the only alternative procedure. The advantages of laser surgery, compared to other methods of correction of urethral strictures, are: the formation of soft, small sized scars (mild impaired elasticity), and a reduced activity of fibroblasts during epithelialization. Moreover, bloodless dissection of the laser has the advantage to limit the use of the urethral catheter in the postoperative period.

Surgical treatment methods of urethral strictures do solve out satisfactorily all the problems related to this disease. Thus, the open surgical treatment outcomes still remain low: recurrence of urethral strictures are found in 5 to 37.4% of cases, impotence development in 34.9 to 80.0% , shortening of the penis in 24% of patients, operative wound suppuration from 10.4 to 16,8% of the cases [53,102,122].

Endourological methods of treatment in urethral strictures, in many cases, replaced complex open surgeries, thus significantly reducing the rate of postoperative complication [105]. However, the recurrence rate of urethral strictures after a primary endoscopic recovery of urethra (visual internal urethrotomy) is 8-23% [119,128]. Endoscopic reconstruction efficiency remains low at 30-50% [23], which leads to incapacity to work, disability and social inadequacy of the patient.

Despite the reported diversity of minimally invasive procedures, the results still remain unsatisfactory in treatment of long strictures and somewhat doubtful for medium length of stricture. Thus, for example, the use of electroresection may generate a deeper scar process, due to a serious electrical injury of the peristrictural tissue, which does not guarantee getting good results [141,143]. There is also an opinion that urethroplasty is indicated in the treatment of long strictures, complicated by false channels, or in complete urethral obliteration [141,144].

Low efficiency of endoscopic correction is, in fact, related to inadequate radicalism of resection of the sclerotic urethral tissue, inadequate management of pre– and postoperative period, the presence of microcirculatory and trophic disorders in the region of the urethral stricture [25].

Therefore, besides the surgical procedures, the pathogenetic therapy must be applied in the treatment of urethral strictures, in order to eliminate edema, inflammation and improve the trophic of the urethral tissue [53,131].

Hitherto, native and foreign researchers have proposed a series of conservative and surgical methods of treatment of urethral strictures in men [109,116,133,145]. However, the indicators of treatment efficiency remain at a fairly low level. Thus, postoperative stricture recurrence is reported in 30-75% of cases, which requires repeated surgical interventions and has an extremely negative impact on quality of life. Additionally, the irrational use of surgical possibilities leads to an increased number of patients with extended urethral strictures, who may need reconstructive surgery [88].

Therefore, treatment of urethral strictures is one of the most acute and contradictory issues in modern urologic surgery. Specialized literature describes the comparative characteristics of different methods of treatment related to this disorder. It is important to note that outcomes in the treatment of urethral strictures, performed by means of the same method, vary from author to author. Yet, there is no unique opinion regarding the optimal choice in surgical treatment of scarred urethral stricture.

Data related to the recurrence and complication rate, surgical methods efficiency, as well as the role of incision and laser coagulation of strictures are quite contradictory [28,119,144]. Also, there have not been set strict guidelines for the use of laser, depending on the size and location of the scarred stricture; there have not been studied the results of late treatment of strictures; and, most importantly, the recurrence rate after procedures performed by means of surgical laser [123,139]. Despite many studies and published scientific researches, which contributed to the improvement of methods of diagnosis and treatment of urethral strictures in men, there are a number of issues which still require being revised [146].

Thus, there is no single solution of this problem regarding the treatment of urethral stricture and obliteration. After hundreds of years, there are still three directions in solving it viz. endoscopic correction, surgical treatment and electrode dilation of the urethra both as independent method or additional to the previous ones. It is currently problematic to create a single perfect method in treatment of both stricture and obliteration of the urethra. Accurate indications for a certain method,in order to restore the urethral permeability, depending on location and length, as well as to improve methods of treatment, would reduce the invasive risks without decreasing the efficiency and may lead to success in each case.

A special place in clinical practice is reported to treatment of postoperative urethral strictures and obliteration of elderly and senile patients [92]. Based on the statistical analysis in recent years, we can conclude indirectly about the number of patients of a certain age. We can mention that about 50% of patients with urethral stricture are over 60. Moreover, due to different associated diseases, prolonged urinary infections after previous interventions involving the urinary tract, this category of patients shows less positive outcomes to any reconstructive intervention. In addition to that, this group of people presents higher complication and postoperative mortality rates. However, there are few rehabilitation activities in people of advanced and senile age, depending on the method applied and postoperative urethral stricture treatment.

Hence, the development of endoscopic surgery has provided new, minimally invasive methods in treatment of infravesical obstruction of different genesis. The choice of method in treatment of infravesical obstruction is determined mainly by two mutually exclusive circumstances: the risk of surgical intervention and restoration of micturition. Often, the selected method must be less offensive compared to the results.

Laser surgery, plasmakinetic, transurethral resection and their combination present a number of advantages compared to open conservative interventions viz. fewer complications, the possibility to apply these methods to patients with severe associated diseases, short-term hospital stay, economic efficiency.

However, despite the listed above advantages, endoscopic interventions, like any other surgeries are associated with complications in 15-30% of cases [63,149,150].

Native and and international scientific sources describe actually the method and technique of endoscopic treatment for infravesical obstruction, while there are fewer studies regarding its complications. One of the perspective directions that contribute to improvement of the treatment quality for infravesical obstruction is the combination of these methods (TURP and Ho Laser; TURP and Vaporization). According to their opinion, in this case, the benefits of one method will compensate the disadvantages for another. Nevertheless, the associated application of minimally invasive procedures does not prevent the incidence of various complications, which are less studied nowadays [119,129,147,148].

The most works of recent years, little or insufficiently describe different types of complications after endoscopic interventions, as well as their criteria of prognosis.

Hitherto, the problem of surgical treatment of infravesical obstruction in endourology has not been highlighted or synthesized by any helpful studies from the Republic of Moldova.

Conclusions

The literature analysis showed that prostate adenoma, sclerosis of bladder neck and prostate, urethral stricture and obliteration can be associated to the disorders that show signs of IVO, characterized by impairment of bladder emptying, difficulties in micturition, retention of the urine and other types of dysuria.

IVO of urinary tract is a polyetiologic and pathological process, characterized by symptoms of lower urinary tract, which despite profound and multilateral study, still remains a serious problem of modern urology. It is a major health and social problem due to its high occurrence in working aged patients, as well as limited preventive measures and progression to severe and fatal complications if no proper treatment is applied.

Recurrence of IVO is the most severe and late postoperative complication due to the scarring process in the urethra and vesico-urethral segment (strictures and obliterations) and the continuous growth of adenomatous tissue mass (real or false BPH recurrence).

The latest results in this field, as well as the scientific and technical progress have changed considerably the traditional methods used in the treatment of many urological diseases causing infravesical obstruction. Modern urology provides plenty of treatment methods for these diseases, particularly, new endoscopic approaches (transurethral and plasmakinetic vaporization, laser Holmium enucleation) and a variety of

their combinations, which display both advantages and disadvantages and compete with traditional ones, considered as basic methods over the past few decades; thus, creating opportunities for an individual approach to ensure a final success of a medical intervention.

A deeper and more complex comparative study is required in terms of application and effectiveness of the techniques and endoscopic transurethral approaches used in the treatment of disorders manifested by IVO, such as BPH, BNS and PS, US and UO, aiming to broaden the range of indications for the implementation of new minimally invasive transurethral methods into practice.

Optimization of problems, related to endoscopic treatment of infravesical obstructive disorders, will help reduce unwanted symptoms of lower urinary tract, fight off complications, including prevention of IVO recurrencies, facilitate recovery and improve patient's quality of life after treatment.

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