

Basi strutturali e funzionali della sindrome genitourinaria

Abbiamo effettuato un esame clinico e morfologico comprendente 70 pazienti in menopausa di età tra i 42 e i 62 anni che soffrivano di disuria e di dolore pelvico cronico. La valutazione della microcircolazione della vescica e della vagina attraverso la debimetria laser Doppler nelle pazienti in menopausa che soffrivano della sindrome genitourinaria ha rivelato dei parametri di microcircolazione ridotti (da 12 a 65%). L'analisi della composizione qualitativa e quantitativa della microflora batterica dell'uretra e della vagina ha rilevato delle anomalie del microbiota di gravità variabile, che potevano essere all'origine dei processi infiammatori infettivi degli organi pelvici inducendo una sindrome di dolore pelvico cronico e una incontinenza. Durante la menopausa, la sindrome genito-urina nelle donne è associata ad uno sviluppo combinato di cambiamenti atrofici della vescica e della mucosa uterina che, per le loro caratteristiche morfologiche, differiscono dai cambiamenti involutivi legati all'età.

L'atrofia (ipoploasia) dell'endometrio e della mucosa vescicale si sviluppa nel contesto di una fibrosi pronunciata e si accompagna ad iperemia e emorragie.

MORPHOLOGY AND PATHOMORPHOLOGY

Structural and Functional Bases of Genitourinary Syndrome of Menopause

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We performed a comprehensive clinical and morphological examination of 70 menopausal patients aged 42-62 years with dysuria and chronic pelvic pain. Assessment of the bladder and vaginal microcirculation by laser Doppler flowmetry in menopausal patients with genitourinary syndrome revealed reduced (by 12-65%) microcirculation parameters. Analysis of the quantitative and qualitative composition of the bacterial microflora of the urethra and vagina revealed abnormalities of microbiota of varying severity, which can be the cause of infectious-inflammatory processes in the pelvic organs leading to chronic pelvic pain syndrome and incontinence. During menopause, the genitourinary syndrome in women is associated with the combined development of atrophic changes in the bladder and uterine mucosa that by their morphological characteristics differ from age-related involutive changes. Atrophy (hypoplasia) of the endometrium and bladder mucosa develops against the background of pronounced fibrosis and is accompanied by hyperemia and hemorrhages.

Key Words: *genitourinary syndrome; menopause; opportunistic microflora; morphology; laser Doppler flowmetry*

Genitourinary syndrome of menopause (GSM) characterized by a complex of disorders in the urogenital system is more often observed in menopause. This term is used in both domestic and foreign studies to denote such urogenital pathologies [1,8,9]. The development of GSM is naturally associated with a reduced level of circulating estrogens and with start of the process of aging [15], which logically substantiates the need for estrogen replacement therapy (systemic or local depending on the symptoms) [4,13]. It is important to note that the decrease in the blood estrogen level can be associated with either natural aging or other causes (including surgical interventions) [6,12].

Clinically, GSM is manifested in urinary disorders and sexual dysfunctions. Women most often complain of vaginal dryness and burning, dyspareunia (pain and/or discomfort during intercourse), recurrent vaginal discharge, contact bleeding, colpoptosis, urination disorders (pollakiuria, nocturia, hyperactive bladder, stress urinary incontinence, mixed form of urinary incontinence, infection of urinary tract, *etc.*). Patients also note lower abdominal pain as well as pain in the urethra, perineum, or vagina.

Despite great interest in the problem of GSM and considerable efforts that are made by researchers and clinicians to develop effective diagnostic and treatment regimens for this condition, it should be noted that this pathology is still not fully understood [8]. This is largely due to insufficient understanding of the structural bases, molecular-biological mechanisms of GSM, and its pathogenesis, which is probably due to

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the heterogeneity of the pathological processes united by this term. This manifests in different severity and heterogeneity of clinical manifestations and different structural and functional changes assessed by the results of pathomorphological, clinical, and instrumental studies. Some authors note that the transformation of urogenital symptoms and signs into a syndrome may create an iatrogenization of menopause, *i.e.* levelling of specific urogenital diagnoses and, accordingly, their targeted treatment, but instead major efforts will be made to develop universal treatment regimens aimed at correction of any disorder in the functioning of the genitourinary system, even when it is not required [14]. In this context, further in-depth study of the structural and functional bases of GSM is needed to develop more effective treatment regimens.

We studied the structural and functional bases of GSM.

MATERIALS AND METHODS

We performed a comprehensive clinical and morphological examination of 70 menopausal women aged 42 to 62 years with dysuria and chronic pelvic pain. Of these, 30 (43%) patients had natural menopause, 37 patients (53%) had surgical menopause, and 4 patients (4 %) had premature ovarian failure with early menstrual dysfunction. At admission, all patients complained of painful urination and nocturia.

In all patients, clinical and instrumental examination including cystoscopy, colposcopy, hysteroscopy, and ultrasound of the pelvic organs was performed. Microcirculation in the bladder and vaginal walls was assessed by laser Doppler flowmetry (LDF; LAKK-02 instrument, LAZMA) [2]. The parameters were recorded in several altered mucosa sites in the bladder, in modified areas of the endometrium, in the internal urethral opening in 4 randomly selected points at a distance of 1-2 cm from the external urethral opening, in the paraurethral area on the front wall of the vagina in 4 randomly selected central and lateral points. In the monitoring mode, the microcirculation index (MI) was recorded that assesses erythrocyte flow per unit time per unit tissue volume; standard deviation (SD), which reflects erythrocyte flow. Both indicators were expressed in perfusion units (perf. units). The coefficient of variation Cv was calculated ($Cv=SD \times MI \times 100$) and the index of efficiency of microcirculation ($IEM=A_{max} LF/A_{max} HF+A_{max} CF$), where A_{max} is maximum amplitude and LF, HF, and CF are slow and fast rhythms and pulse fluctuations. The parameters of LDF-grams were compared with those of 15 healthy patients constituting the control group [2].

Quantitative and qualitative assessment of microflora in the vaginal and urethral mucosa was per-

formed by real-time PCR. The result was presented by the absolute amount of microbial DNA and relative content of genetically related groups of microorganisms in the total bacterial mass. The relative number of individual types of bacteria was expressed as the difference of the decimal logarithms of the relative content of the corresponding microorganism and the total bacterial mass.

For pathomorphological analysis, bladder mucosa specimens taken from the hemorrhagic foci, bladder trigone, and urethral and lateral surfaces, as well as specimens of endometrium and endocervix collected by targeted separate curettage were fixed in 10% neutral formalin, and after standard processing embedded in paraffin. Paraffin sections (3-5 μ) were stained with hematoxylin and eosin and after van Gieson. To prepare semithin sections, the tissue specimens were fixed in 4% paraformaldehyde and 1% OsO_4 , embedded in a mixture of epon and araldite. Semithin (1 μ) sections were sliced on an LKB-III ultratome and stained with 1% azure II. The preparations were examined under a Leica DM 4000B universal microscope and photographed using a Leica DFC 320 digital camera and a Leica QWin V3 software.

RESULTS

Assessment of the bladder microcirculation in patients with GSM revealed a decrease in MI, SD, and IEM by 66, 12, and 57.4% ($p<0.05$), respectively, in comparison with healthy individuals (Table 1). In the vagina, MI, SD, and IEM were decreased by 64, 12, and 35%, respectively ($p<0.05$) (Table 1). Analysis of LDF-grams allowed us to identify the stasic-hemodynamic type of microcirculation [2].

Analysis of microflora in patients with GSM excluded specific infections. The study of the quantitative and qualitative composition of the microflora showed microbiota abnormalities of different degrees in the vaginal and urethral mucosa (Table 2). Opportunistic microflora was presented by *E. coli*, *Staphy-*

TABLE 1. Parameters of LDF of the Bladder and Vagina in Patients with GSM ($M \pm m$)

Parameter	MI	SD	Cv, %	IEM
Bladder	9.75±0.41*	6.45±0.15*	31.48±0.49*	0.78±0.05*
Vagina	10.3±0.21*	6.45±0.45	33.37±0.28*	0.89±0.21
Normal value	28.5±0.8	7.31±0.28	25.65±0.36	1.36±0.10

Note. MI: index of microcirculation; SD: standard deviation reflects erythrocyte flow (perfusion units); Cv: coefficient of variation; IEM: index of microcirculation efficiency. * $p<0.05$ in comparison with normal values.

TABLE 2. Microflora of the Vagina and Urethra in Patients with GSM

Microorganisms	Colonization of vagina, LgCFU/g	Colonization of urethra, LgCFU/g
<i>Lactobacillus spp.</i>	5.2±1.0	4.8±1.1
<i>Candida</i>	6.4±1.6	5.8±1.2
<i>Streptococcus faecalis</i>	5.4±0.8	5.4±0.9
<i>Streptococcus agalactae</i>	5.1±0.4	5.3±0.3
<i>Streptococcus haemoliticus</i>	6.0±0.2	5.8±0.3
<i>Streptococcus saprophyticus</i>	4.2±0.3	4.5±0.2
<i>E. coli</i>	5.3±0.2	5.1±0.3
<i>Enterococcus spp.</i>	6.0±0.1	6.0±0.2
<i>Enterobacter aerogenes</i>	5.8±1.0	5.5±0.1
<i>Corynebacterium</i>	4.7±1.0	—
<i>Citrobacter fr.</i>	3.9±0.1	—
<i>Staphylococcus aureus</i>	4.7±0.7	5.2±0.3
<i>Streptococcus epidermis</i>	4.6±0.1	4.9±0.1
<i>Staphylococcus spp.</i>	4.5±1.0	5.0±1.3
<i>Streptococcus spp.</i>	—	3.9±0.2

lococcus aureus, *Streptococcus faecalis*, *Candida*, *Corynebacter spp.* Increased colonization of facultative microflora in patients was accompanied by a decrease in *Lactobacillus spp.* colonization. The pronounced decrease in the number of lactobacilli contributing to dysbiotic changes was paralleled by atrophy of the vaginal mucosa.

Similar changes in the microbiota of vaginal and urethral mucosa during menopause were also reported by other researchers [11]. A decrease in estrogen levels is accompanied by a decrease in the prevalence of *Lactobacillus*, alkalization of the vaginal secretion (pH>5.0), and the growth of gram-negative flora, including fecal flora, streptococcus group B, staphylococci, coliforms, including vaginal infections and urinary tract infections [9]. Patients were diagnosed bacterial vaginosis, desquamative and candidal vaginitis. These processes were associated with the growth of several types of microorganisms that could potentially cause infectious and inflammatory processes in the pelvic organs in women and lead to the development of pelvic pain syndrome accompanying GSM.

Gynecological examination and colposcopy in all patients detected atrophy of the vaginal mucosa (pH 5.6-6.0). Ultrasound examination of the pelvic organs showed signs of chronic cystitis in all patients, endometrial atrophy in 43 (61.4%) non-operated patients, small non-perfused fibroids in 2 (2.85%) patients, endometrial polyps in 10 patients (14.28%), and intrauterine fluid in 11 (15.7%) patients.

Endoscopic studies revealed similarity of the macroscopic picture of pathological atrophy of uterine and

bladder mucosa. In contrast to climacteric (naturally developing) atrophy of the bladder mucosa and endometrium characterized by thinning and pale color of the mucosae, the “pathological” atrophy was characterized by damaged purple thinned mucosa with multiple hemorrhages, signs of secondary infection in both the uterus and bladder.

In non-menopausal women with urogenital disorders, epithelial metaplasia in the Lietho triangle area was detected during cystoscopy. All examined patients had increased vascular pattern (100%). In many cases, ulcerative changes (80%), multiple hemorrhages (67.1%), small polypoid vegetation (21.4%) were seen. We also observed deformities of the bladder cavity, protrusion of the posterior wall (48.6% of cases), swelling of the cervical mucosa in the area of the Lietho triangle (38.6%), salt impregnation (20% of cases).

Hysteroscopy showed signs of infection in the atrophied mucosa with moderate hyperemia (in 80% of cases) and small hemorrhages (100%); in some patients with polyps (20%), the mucosa were without fibrin deposits. Significant decrease in the size of the uterus (up to 5.6 cm) with age in non-operated patients is worthy of note. Deformation of the uterus is noted in 16.7% of cases.

During pathomorphological analysis of the endometrium in patients with premature (pathological) menopause, its hypoplastic changes were most often detected, the stromal component prevailed over the glandular one (Fig. 1, a). Some areas in this endometrium could be characterized as “non-functioning”. En-

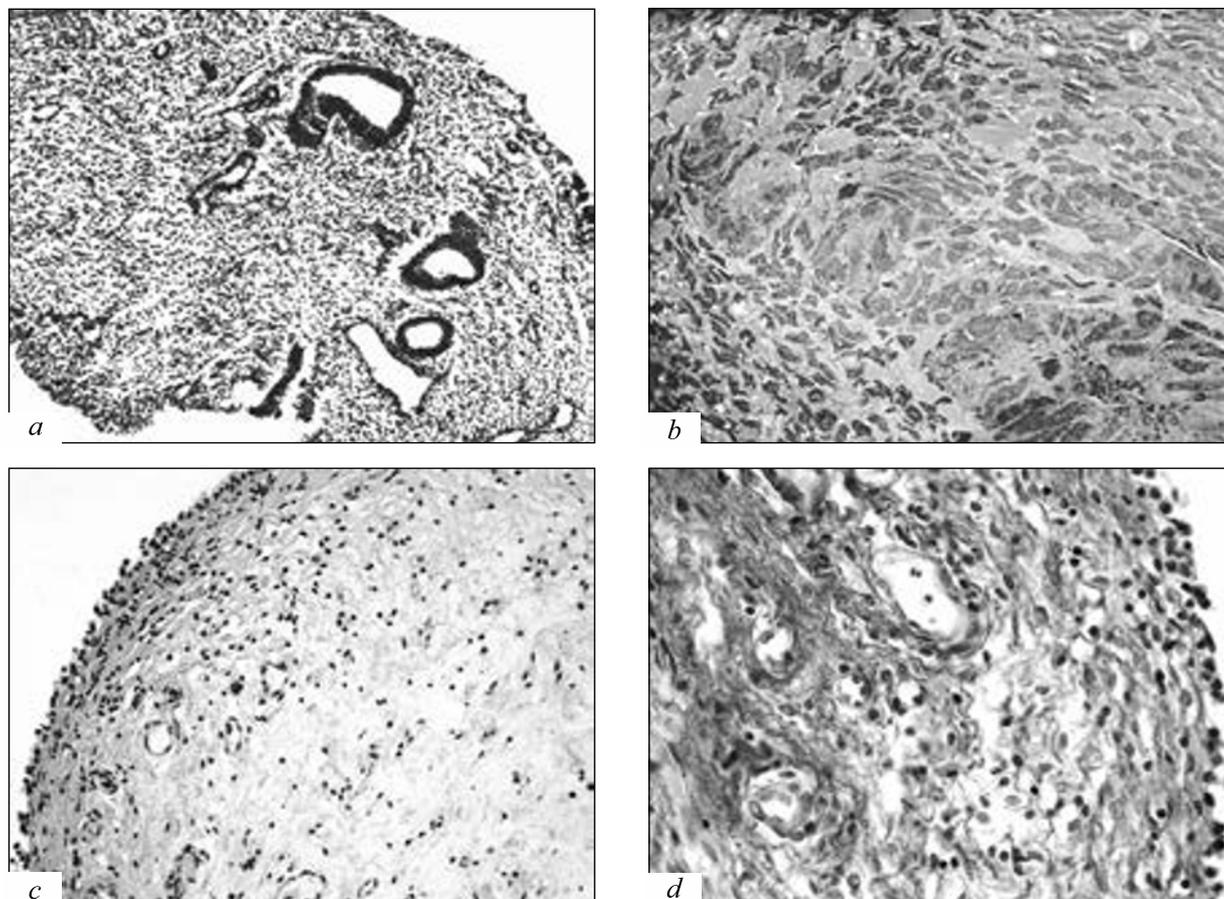


Fig. 1. Morphological changes in the endometrium and bladder mucosa in GSM. Hematoxylin and eosin staining, $\times 200$ (a, c); azure II staining, $\times 1000$ (b); van Gieson staining, $\times 400$ (d). a) Endometrial hypoplasia, stromal component prevails over glandular one. b) Obliteration of endometrial glands. Semithin section. c) Unevenly desquamated urothelium; moderate lymphohistiocytic infiltration of the subepithelial zone of the lamina propria. d) Pronounced fibrosis of the lamina propria of the bladder mucosa; uneven vascular plethora. van Gieson staining, $\times 400$.

ometrium often looked compact, but in some cases, loosening of the endometrium as a result of moderate edema was noted. Endometrial glands in these areas were located unevenly. Some endometrial glands were lined with secretory epithelium, while others were collapsed with obliterated lumen and epithelial metaplasia. Obliterated glands underwent fibrosis and fibrinoid imbibition (Fig. 1, b). It should also be noted that the obliteration of the endometrial glands was most often accompanied by de-epithelialization of the endometrium (extensive areas lacking epithelial cells).

In postmenopausal women, endometrial atrophy was associated in some cases with senile endometritis, ulceration of the surface epithelium, diffuse inflammatory infiltration and hemorrhagic imbibition of thinned endometrium. In 11 (15.7%) cases, fibrous-glandular polyps of indifferent and retrogressive types were diagnosed. In 4 (5.7%) cases, “synechiae” represented by the fibrous stroma and compressed small slit-like glands with an indifferent epithelium were detected in the scrapes of uterine cavity.

Endometrial atrophy due to the age-related decrease in the estrogen level, according to some authors, is most often detected in postmenopausal bleeding (up to 52% of all cases of uterine bleeding) [5,7], which is a serious clinical problem.

Menopausal cystitis with atrophy of urothelium was detected in biopsy specimens of the bladder mucosa. Uneven thinning of the transitional epithelium with flattening of the facet and intermediate cells, as well as degenerative changes in the urothelium with surface and deep erosion were seen. In these areas, urotheliocytes were partially or completely desquamated (Fig. 1, c). In some patients, the foci of squamous epithelial metaplasia were observed in biopsy specimens of bladder.

In the lamina propria and in the submucosal layer, inflammatory infiltration was moderately pronounced and presented mainly by mononuclear cells (lymphohistiocytic infiltration). In the lamina propria, small focal accumulations of lymphocytes were observed with their penetration into the urothelium. Blood vessels,

were unevenly plethoric, lymphostasis were found (in some cases we can speak about lymphangiogenesis and hemorrhages). Moderate edema of lamina propria should also be noted.

In all cases, marked fibrosis of the lamina propria in the bladder mucosa (Fig. 1, *d*) attested to the chronic course of the pathological process. In some patients, pronounced fibrosis was combined with marked diffuse lymphoid infiltration of the subepithelial zone.

As was shown in many studies, atrophic changes in urogenital mucosae in the menopausal period develop as a result of estrogen deficiency and are a cause of incontinence in women [10]. The syndrome of chronic pelvic pain can also be the consequence of pronounced urogenital disorders in postmenopausal women. Structural and functional changes in GSM are described mainly as atrophic and dysfunctional, determined by thinning of the mucosa of the urinary tract and vagina, involution of the external genital organs, reduction of their flexibility, elasticity, strength, and increased sensitivity to damaging effects (including mechanical) [3]. The most marked manifestations of GSM include vulvovaginal atrophy and cystourethral atrophy, but they are only a part of the whole spectrum of structural and functional changes detected in this state [3]. Degenerative and atrophic changes in the mucosa in urogenital organs and changes in epithelial-connective tissue relationships can be both transient and progressive (irreversible), which should be determined and taken into account when choosing the therapy for urogenital pathology.

Thus, in menopausal women with GSM, microcirculation disturbances in the vaginal and bladder mucosa develop against the background of dysbiotic changes. The syndrome of chronic pelvic pain in women is determined by atrophic changes in the mucosa of the vagina, urethra, bladder, and uterus that differ by their morphological characteristics from physiological age-related involutive changes. Atrophy (hypoplasia) of the endometrium and bladder mucosa develops against the background of pronounced fibrosis and is accompanied by hyperemia and hemorrhages.

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