Clinical and morphological efficacy and safety of laser remodeling therapy in patients with gsm

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SUMMARY (Abstract)

Study Objective: To evaluate the clinical and morphological efficacy and safety of laser remodeling therapy in patients with genitourinary syndrome of menopause.Study Design: An open, multicentre, comparative, independent, prospective clinical and morphological study.Materials and Methods: This study included 114 patients aged 52.04 ± 1.48 years with a verified diagnosis of postmenopausal atrophic vaginitis who provided voluntary informed consent to participate in this study. Patients were divided into two study groups. To treat vulvovaginal atrophy, patients in group I (n = 59) administered intravaginal suppositories containing 0.5 mg of estriol, 1 suppository per day for the first 4 weeks and then 1 suppository 2 times a week for 8 weeks. The total course of topical hormone therapy was 3 months. In group II (n = 55) all patients underwent laser remodeling of the vulvovaginal region using the SmartXide2 V2LR Monalisa Touch laser system (DEKA, Florence, Italy) in the mode of 40/1400/1000/1 ST/DP for 3 sessions in total with a 1-month interval. The control group (n = 30) included women of similar age who did not have a verified diagnosis of postmenopausal atrophic vaginitis, N95.2.In all patients, tissue specimens (up to 3 mm in diameter) were obtained by punch biopsy at baseline and 3 months after the treatment. Morphological examination was carried out using the light microscopy, which was preceded by standard preparation of biopsy specimens (fixation in neutral formalin, histological processing, staining with hematoxylin and eosin, embedding). Two tissue samples, namely from the anterior and posterior vaginal walls, were obtained in each patient at two time points, i.e., prior to and 3 months after the treatment for GSM. A total of 516 vaginal wall histopathological examinations (114 patients * 2 biopsy specimens * 2 time points + 30 * 2 biopsy specimens in the morphological control group) were performed using light microscopy (hematoxylin and eosin staining) and immunohistochemistry. The results of the IHC with anti-type IV collagen, anti-VEGF-A, and anti-CD-31 antibodies were scored based on the percentage of stained cells and the staining intensity. For objectification, the clinical manifestations of GSM in study patients were rated using a 5-point D. Barlow scale and the vaginal health index was calculated (G. Bachmann, 1995).RESULTS: The mean age of patients in groups I and II was 51.85 ± 1.37 and 52.1 ± 1.26 years, respectively. The mean postmenopausal period was 2.44 ± 0.86 years and 2.26 ± 0.84 years in group I and group II, respectively. The mean time from the onset of clinical manifestations of vulvovaginal atrophy was 2.32 ± 0.84 years in group I and 2.26 ± 0.84 years in group II.This study demonstrated similar clinical and morphological efficacy of the two treatments: 3 months after the therapy the expression of this marker significantly increased by 1.19 (2.19 ± 0.57 versus 2.61 ± 0.5; p<0.001) and 1.14 times (2.29 ± 0.63 versus 2.6 ± 0.49; p<0.001) in groups I and II, respectively; as well there were 1.17-fold (13.6 ± 3.8 versus 15.97 ± 4,

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p<0.001) and 1.3-fold (12.75 ± 4.26 versus 16.69 ± 4.3, p<0.001) increases in the expression of the above marker of angiogenesis and 1.25-fold (14.63 ± 9.01 versus 18.33 ± 10.83, p<0.001) and 1.22-fold (16.78 ± 10, 13 versus 20.51 ± 11.50, p<0.001 respectively) increases in the expression of type IV collagen. In groups I and II the vaginal health scores using the G. Bachmann scale significantly increased 1.27-fold (2.98 ± 0.75 vs. 3.78 ± 1.15; p = 0.006) and 1.17-fold (3.14 ± 0.65 vs. 3.66 ± 1.58; p=0.006), respectively, 3 months after the treatment for GSM.CONCLUSIONS: In summary, in this study the laser remodeling treatment for symptoms of vulvovaginal atrophy using the SmartXide2 V2LR Monalisa Touch fractional CO2 laser (DEKA, Florence, Italy) was shown to have similar clinical and morphological efficacy to topical hormone therapy in improving the parameters of angio- and collagenogenesis in postmenopausal patients: following the treatment the expression of the VEGF-A marker significantly increased 1.19- and 1.14-fold along with increases in the expression level of CD-31 of 1.17- and 1.3-fold, in the expression of type IV collagen of 1.25- and 1.22-fold and in the vaginal health scores using the G. Bachmann scale of 1.27- and 1.17-fold (p<0.05).

Key words: vulvovaginal atrophy, genitourinary syndrome of menopause, laser treatment, pathogenesis, clinical and morphological efficacy

Background

It is important to describe GSM in terms of its clinical manifestations. Thus, vulvovaginal atrophy occurs due to impairment of the histoarchitecture and thinning of the epithelial compartment of the vulvovaginal region, both associated with hypestrogenism. Moreover, the insufficient effect of estrogens on the genitourinary tissues translates into the decreased levels of glycogen in the cells of the vaginal mucosa; and since glycogen is a nutrient medium for beneficial lactic acid bacteria, the population of these microorganisms decreases significantly. As a consequence, the impaired production of lactic acid by these bacteria results in an inevitable shift to alkaline vaginal pH, decreased colonization resistance and development of infectious diseases. These pathological changes lead to a number of clinical manifestations, such as dyspareunia, dysorgasmia, social disadaptation, dysuria, urinary incontinence, etc. [4]. In view of this it is important to look into the underlying causes of vulvovaginal atrophy, namely, the structure of the pathogenetic cascade of GSM.

In addition to disorders of immune homeostasis and proliferation of the mucous membranes of the urogenital region, hypoestrogenism also affects the histoarchitecture of the underlying tissues. Thus, a decrease in estrogen concentration triggers a shift in the ratio of type I and type III collagen towards the "old" type I collagen, as well as structural disorganization of elastic fibers [7, 8, 9].

It should be noted that the age-related decrease in estrogen level triggers a number of disorders, ranging from impaired immune homeostasis to structural disorders of the connective tissue compartment of the pelvic organs, which in combination contribute to vulvovaginal atrophy.

A number of molecular disorders serving as predictors of atrophic changes in the urogenital region play an important role in the pathogenetic cascade of GSM. Thus, the vascular endothelial growth factor (VEGF-A), which is one of the most important markers, prevents the endothelial dysfunction and hypoperfusion of the tissues in the vulvovaginal region. Binding to the VEGFR-2 receptor VEGF-A exerts a number of important biological effects, such as the increase in the vascular permeability, activation of endotheliocyte proliferation and increase in resistance of endothelial cells to reactive oxygen species, which have a pronounced cytotoxic effect [10].

Besides VEGF, there is another predictor of endothelial dysfunction, namely CD-31 or endothelial cell adhesion molecule-1 (PECAM-1). This marker is a receptor of the plasma membrane of endotheliocytes. This makes it a molecular-specific predictor, the content of which potentially reflects the perfusion level of tissues in the urogenital region [11].

Therefore, based on the above, GSM is considered to be one of the most urgent problems of modern gynecology. It should be noted that there is an ongoing active search for specific molecular predictors, the determination of the expression of which will allow not only to objectively verify the degree of urogenital atrophy, but also to demonstrate the efficacy of GSM treatment over time.

Study objective

To evaluate the clinical and morphological efficacy and safety of laser remodeling therapy in patients with genitourinary syndrome of menopause.

Study design

An open, multicentre, comparative, independent, prospective clinical and morphological study.

Materials and methods

190 postmenopausal patients were randomized in this study. 25 patients did not meet the inclusion criteria, 32 non-compliant individuals did not follow the treating physician's recommendations, and 19 subjects withdrew from the study due to a change of residence. This study included a total of 114 patients with a verified diagnosis of postmenopausal atrophic vaginitis (ICD N.95.2) aged 52.04 ± 1.48 years who recieved treatment at the clinical site of the Department of Obstetrics and Gynecology with a course of perinatology of the Peoples' Friendship University of Russia and provided their voluntary informed consent for enrollment in this study. The overall cohort was divided into two study groups. To treat vulvovaginal atrophy, patients in group I (n = 59) administered intravaginal suppositories containing 0.5 mg of estriol, 1 suppository per day for the first 4 weeks and then 1 suppository 2 times a week for 8 weeks. Thus, the total

course of topical hormone therapy was 3 months. In group II (n = 55) all patients underwent laser remodeling of the vulvovaginal region using the SmartXide2 V2LR Monalisa Touch laser system (DEKA, Florence, Italy) in the mode of 40/1400/1000/1 ST/DP for 3 sessions in total with a 1-month interval. Thus, the total course of laser remodeling treatment was 3 months.

The control group (n = 30) included women of similar age who did not have a verified diagnosis of postmenopausal atrophic vaginitis, N95.2.

For objectification, the clinical manifestations of genitourinary syndrome of menopause in study patients were scored using a 5-point D. Barlow scale. Based on the above signs, vulvovaginal atrophy was objectified by calculating the Vaginal Health Index (G. Bachmann, 1995).

In all patients, tissue specimens (up to 3 mm in diameter) were obtained by punch biopsy at baseline and 3 months after the treatment. Morphological examination was carried out using the light microscopy, which was preceded by standard preparation of biopsy specimens (fixation in neutral formalin, histological processing, staining with hematoxylin and eosin, embedding). Two tissue samples, namely from the anterior and posterior vaginal walls, were obtained in each patient at two time points, i.e., prior to and 3 months after the treatment for GSM. A total of 516 vaginal wall histopathological examinations (114 patients * 2 biopsy specimens * 2 time points + 30 * 2 biopsy specimens in the morphological control group) were performed using light microscopy (hematoxylin and eosin staining) and immunohistochemistry at the Federal State Budgetary Scientific Institution "A.P. Avtsyn Research Institute of Human Morphology" (director, head of the laboratory of clinical morphology – honored scientist of the Russian Federation, corresponding member of the Russian Academy of Sciences, D. Sci. (Med.), professor L.M. Mikhaleva).

The results of the IHC with anti-type IV collagen, anti-VEGF-A, anti-CD-31 antibodies were scored based on the percentage of stained cells and the staining intensity. A histoscore was calculated using the following formula: HS=Σ (Pi × i), where Pi is the percentage of stained cells for each intensity (from 0% to 100%), i is the intensity of staining scored from 0 to 3 (0 – no staining; 1 – weak [light brown]; 2 – moderate [brown]; 3 – strong [dark brown] staining).

To analyze the findings from this morphological and immunohistochemical study, the study groups were compared with the morphological comparison group (n = 30), which included patients who did not have a verified diagnosis of postmenopausal atrophic vaginitis (N95.2). In patients from the morphological comparison group (n = 30) without signs of vulvovaginal atrophy biopsy specimens were obtained during a pelvic-floor reconstructive surgery.

For statistical analysis, the results were processed using IBM SPSS v.23.0 and StatTech software. The arithmetic means, standard deviations and errors of means were calculated. Statistical significance of differences was assessed using non-parametric criteria. i.e., the Mann-Whitney U-test and the Kruskal-Wallis H-test.

To compare the study groups, the t-test was used with a significance level of $p < 0.05$

Results

The mean age of patients in the study cohort (n = 114) was 52.04 ± 1.48 years with the mean age of 51.85 ± 1.37 and 52.1 ± 1.26 years in groups I and II, respectively. There were no statistically significant differences between the study groups and the control group (mean age = 51.17 ± 1.62) (p = 0.212). The mean postmenopausal period was 2.44 ± 0.86 years and 2.26 ± 0.84 years in group I and group II, respectively, with no statistically significant differences in postmenopausal period between the study groups and the control group (p = 0.285). In all patients in the study cohort data regarding the duration of clinical manifestations of GSM were collected. Thus, the mean time from the onset of clinical manifestations of vulvovaginal atrophy was 2.32 ± 0.84 years in group I and 2.26 ± 0.84 years in group II with no statistically significant differences between the groups (p = 0.827).

In groups I and II the vaginal health scores using the G. Bachmann scale significantly increased 1.27-fold (2.98 ± 0.75 vs. 3.78 ± 1.15; p = 0.006) and 1.17-fold (3.14 ± 0.65 vs. 3.66 ± 1.58; p=0.006) and 1.06-fold (2.98 ± 0.75 vs. 3.17 ± 0.79; p=0.013) and 1.0032-fold (3.14 ± 0.65 vs. 3.15 ± 1.04; p=0.013), respectively, 3 and 6 months after the treatment for GSM. Meanwhile, the intensity of clinical manifestations of GSM (based on the analysis of the complaint rate in the study cohort) significantly decreased 1.84-fold (2.95 ± 1.24 vs. 1.6 ± 1.04; p < 0.001) and 2.15-fold (2.93 ± 1.14 vs. 1.36 ± 0.91; p<0.001) and 1.48-fold (2.95 ± 1.24 vs. 2 ± 0.8; p<0.001) and 1.97-fold (2.93 ± 1.14 vs. 1.49 ± 0.96; p<0.001) in groups I and II, respectively, 3 and 6 months after the treatment for vulvovaginal atrophy. This study revealed significant differences in the D. Barlow scores of the study groups and the control group both at baseline (2.95 ± 1.24 and 2.93 ± 1.14 vs. 0.07 ± 0.25; p<0.001) and 3 months (1.6 ± 1.04 and 1.36 ± 0.91 vs. 0.07 ± 0.25; p<0.001) and 6 months (2 ± 0.8 and 1.49 ± 0.96 vs. 0.07 ± 0.25; p<0.001) after the treatment.

The baseline levels of VEGF-A expression in groups I and II were 2.19 ± 0.57 and 2.29 ± 0.63, respectively (Fig. 2 and 3). 3 months after the treatment, the expression of this marker significantly increased by 1.19 (2.19 ± 0.57 versus 2.61 ± 0.5; p<0.001) and 1.14 times (2,19 ± 0.63 versus 2.6 ± 0.49; p<0.001) in groups I and II, respectively (Fig. 1, 2 and 3).

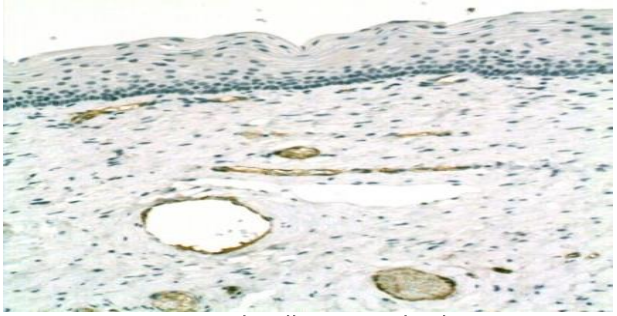


Figure 1 — Vaginal wall (IHC method; anti-VEGF-A antibodies, counterstaining with hematoxylin, ×200): a – no signs of vulvovaginal atrophy

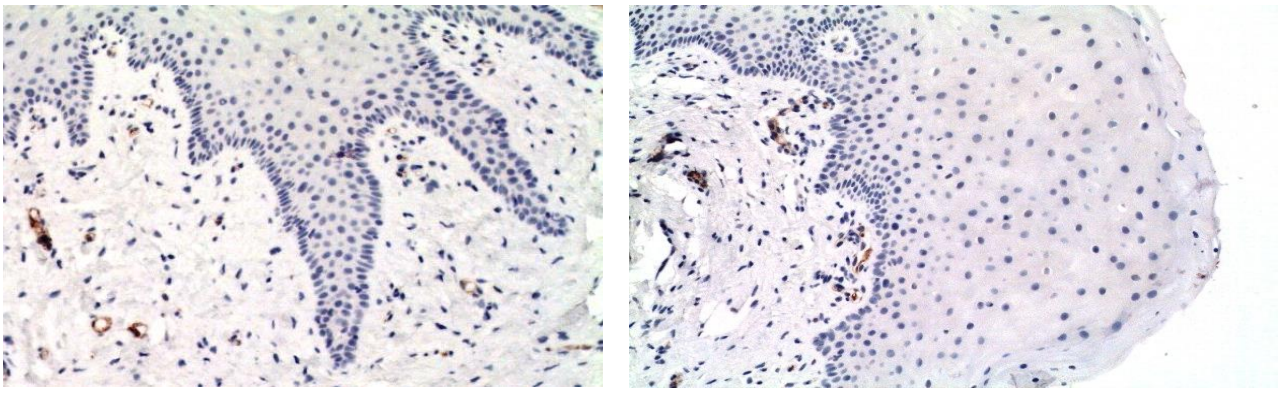


*Figure 2 — Vaginal wall (IHC method; anti-VEGF-A antibodies, counterstaining with hematoxylin, ×200): **a** – before topical hormone therapy b – 3 months after topical hormone therapy*

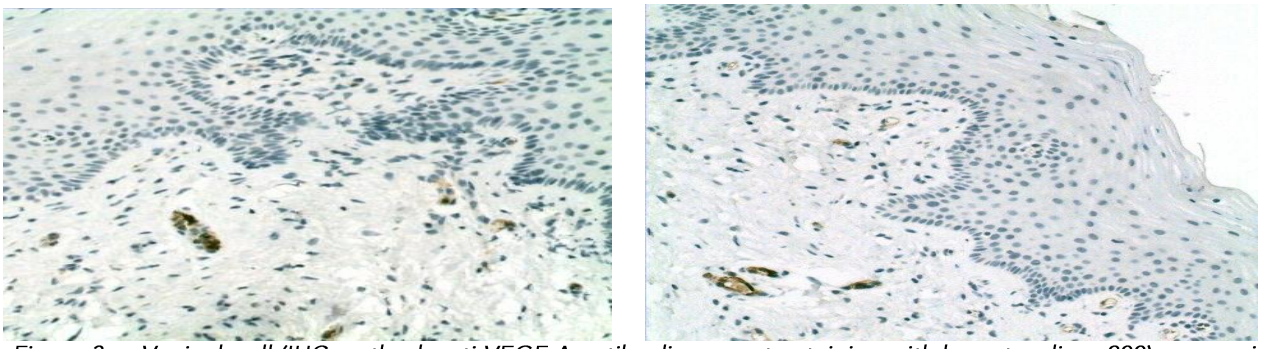
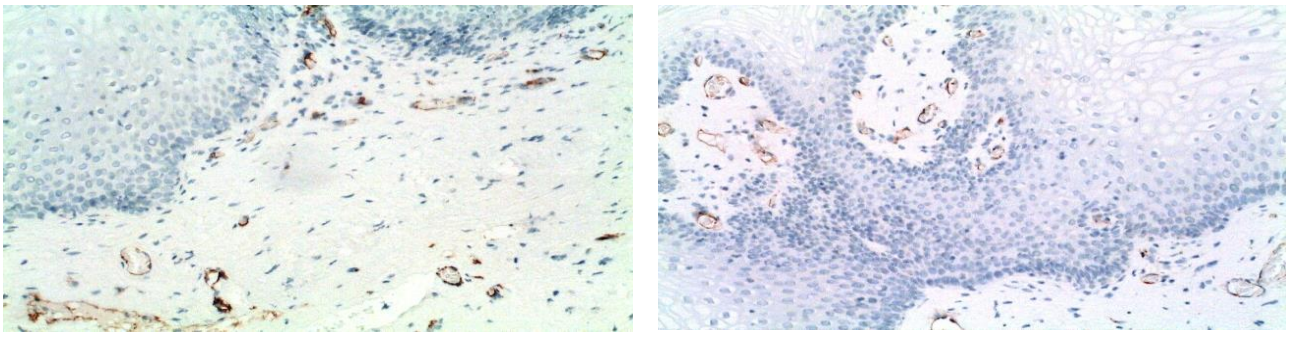


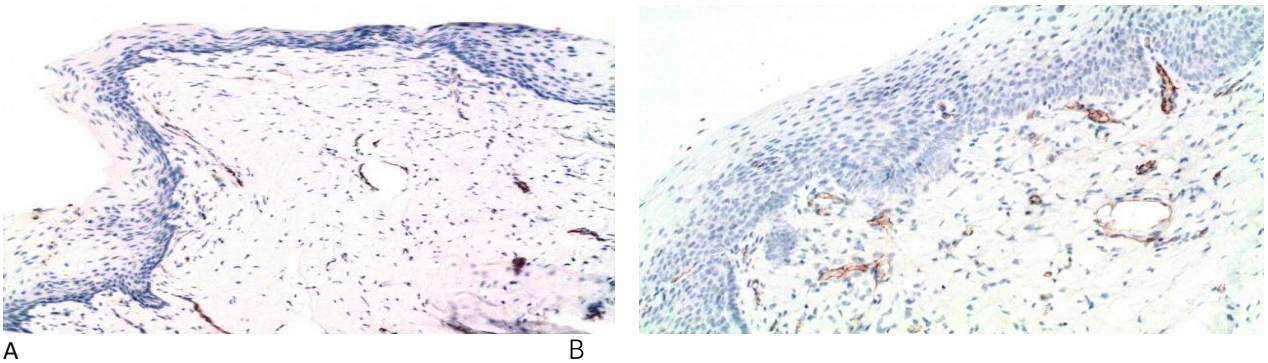
Figure 3 — Vaginal wall (IHC method; anti-VEGF-A antibodies, counterstaining with hematoxylin, ×200): a – no signs of vulvovaginal atrophy, b – vulvovaginal atrophy before laser treatment; c – 3 months after laser treatment

However, in addition to VEGF-A, CD-31 is an equally important proangiogenic marker, the decrease in expression of which contributes to perfusion disorders of the vaginal wall. This marker also plays an important role in the pathogenetic cascade of vulvovaginal atrophy. The baseline expression of the CD-31 marker in the study groups differed downward and was 13.6 ± 3.8 (Fig. 4) and 12.75 ± 4.26 (Fig. 5) in groups I and II, respectively. Following the treatment, the expression of the above angiogenesis marker increased in both groups: 1.17-fold (13.6 ± 3.8 versus 15.97 ± 4, p<0.001) (Fig. 4) and 1.3-fold (12.75 ± 4.26 vs. 16.69 ± 4.3, p<0.001) (Fig. 5).



A B

*Figure 4 — Vaginal wall (IHC method; anti-CD-31 antibodies, counterstaining with hematoxylin, ×200): **a** – before topical hormone therapy, b – 3 months after topical hormone therapy*



A B

*Figure 5 — Vaginal wall (IHC method; anti-CD-31 antibodies, counterstaining with hematoxylin, ×200): **a** – before laser treatment, b – 3 months after laser treatment*

In patients without signs of vulvovaginal atrophy, the expression of type IV collagen was 35 ± 2.62 (Fig. 6). Meanwhile, the expression of the above marker in groups I and II was found to be lower, i.e., 14.63 ± 9.01 (Fig. 7) and 16.78 ± 10.13 (Fig. 8). However, following the treatment for GSM there was a positive trend observed in the expression of type IV collagen in both groups. Thus, the expression of this marker increased 1.25-fold (14.63 ± 9.01 versus 18.33 ± 10.83, p<0.001) (Fig. 7) and 1.22-fold (16.78 ± 10.13 versus 20.51 ± 11.50, p<0.001) (Fig. 8) in groups I and II, respectively.

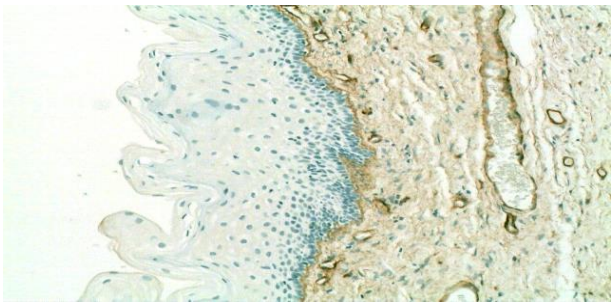
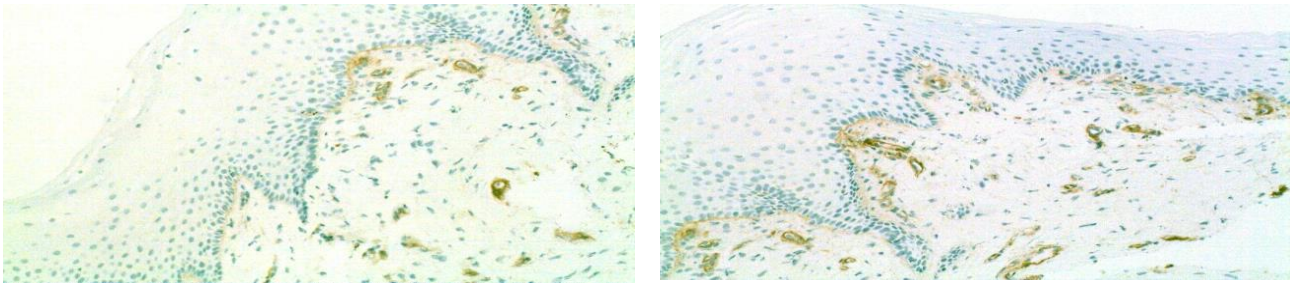
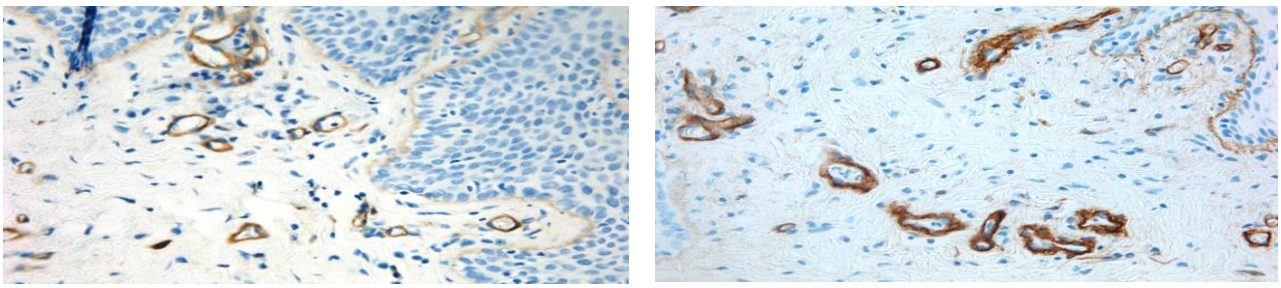


Figure 6 — Vaginal wall (IHC method; anti-type IV collagen antibodies, counterstaining with hematoxylin, ×200), no signs of vulvovaginal atrophy



A B

*Figure 7 — Vaginal wall (IHC method; anti-type IV collagen antibodies, counterstaining with hematoxylin, ×200): **a** – before topical hormone therapy, b – 3 months after topical hormone therapy*



A B

*Figure 8 — Vaginal wall (IHC method; anti-type IV collagen antibodies, counterstaining with hematoxylin, ×400): **a** – before laser treatment, b – 3 months after laser treatment*

Discussion

Genitourinary syndrome of menopause is one of the main postmenopausal disorders associated with natural age-induced hypoestrogenism and has an extremely wide range of incidence rates and clinical symptoms. Changes in the genitourinary tract associated with the end of the reproductive period of life and with the inevitable aging represent one of the most urgent problems of modern gynecology [9]. To describe estrogen-dependent age-related changes affecting the vulva, vagina, urethra and bladder, a new term was adopted, i.e., a genitourinary syndrome of menopause, GSM (IMS, 2014) [9]. As it follows from the above, the use of topical hormone therapy to treat GSM is absolutely pathogenetically justified, and the topical estriol preparations demonstrate high efficacy and safety. However, it is worth noting that the "gold standard" treatment for GSM also has the disadvantage that within 1–3 months after discontinuation of topical estrogens one should expect the recurrence of de novo symptoms [10]. Undoubtedly, this aspect not only merits the attention of the global gynecological community, but also sets forth a search trajectory for new therapeutic approaches that can "reverse" the progressive symptoms of vulvovaginal atrophy and "block" GSM at the level of its pathogenetic patterns [11].

It is important to mention the findings of a large meta-analysis by Filippini M. et al. (2022), who studied the effects of laser remodeling treatment on the clinical manifestations of GSM. There was a clear decreasing trend observed in the clinical symptoms of all major subjective patterns of vulvovaginal atrophy: dryness -5.15 (95% CI: -5.72, -4.58; p < 0.001; I2: 62%; n = 296), dyspareunia -5.27 (95% CI: -5.93, -4.62; p < 0.001; n = 296), itching -2.75 (95% CI: -4.0, -1.51; p < 0.001; I2: 93%; n = 281), burning sensation -2.66 (95% CI: -3.75, -1.57; p < 0.001; n = 296) and dysuria -2.14 (95% CI: -3.41, -0.87; p < 0.001; n = 281). In addition, there was a pronounced increase noted in the female sexuality index, which showed a mean difference of 10.8 (95% CI: 8.41,13.37; p < 0.001; n = 273) [12].

In this regard, it is important to mention an extremely promising, although not reflected in the world clinical guidelines, treatment option for GSM, namely, the laser remodeling of the vulvovaginal region. Despite the fact that the use of laser technologies in gynecological practice is quite a novelty, the method of laser tissue remodeling has

been used in cosmetology for a long time. Currently, the fractional microablative CO_2 laser is one of the most technically advanced and state-of-art devices. Based on the above, it is important to note that the use of the fractional CO_2 laser in patients with verified GSM demonstrated high efficacy and safety with no adverse effects.

Thus, we have looked into the new understandings of the mechanisms of pathogenesis and the main therapeutic approaches to the treatment of vulvovaginal atrophy, both "classic" and innovative and extremely promising. It should be noted that in light of the fact that topical hormone therapy and a course of laser remodeling procedures demonstrated similar efficacy in relieving symptoms of GSM, the method of laser remodeling of the vulvovaginal region can be used as the first-line treatment in patients with contraindications to topical estrogen therapy or hormonophobia.

Conclusions

In summary, in this study the laser remodeling treatment for symptoms of vulvovaginal atrophy using the SmartXide2 V2LR Monalisa Touch fractional CO2 laser (DEKA, Florence, Italy) was shown to have similar clinical and morphological efficacy to topical hormone therapy in improving the parameters of angio- and collagenogenesis in postmenopausal patients: following the treatment the expression of the VEGF-A marker significantly increased 1.19- and 1.14-fold along with increases in the expression level of CD-31 of 1.17- and 1.3-fold, in the expression of type IV collagen of 1.25- and 1.22-fold and in the vaginal health scores using the G. Bachmann scale of 1.27- and 1.17-fold ($p<0.05$).

The use of the SmartXide2 V2LR Monalisa Touch fractional CO2 laser (DEKA, Florence, Italy) in treatment of GSM is pathogenetically justified and is an effective treatment option in postmenopausal patients with contraindications to topical hormone therapy.

Disclosure of interest

The authors declare that they have no competing interests.

Authors' contribution

The authors declare the compliance of their authorship according to the international ICMJE criteria.

All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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