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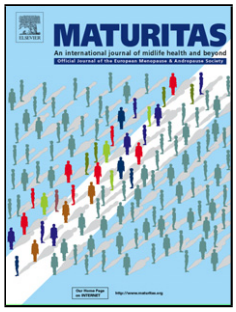

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LASER THERAPY FOR THE GENITOURINARY SYNDROME OF MENOPAUSE. A SYSTEMATIC REVIEW AND META-ANALYSIS

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Highlights

- Intravaginal laser-therapy appears promising for the treatment of GSM
- Available studies consistently indicate alleviation of all GSM symptoms
- Sexual function and quality of life may improve clinically meaningful
- Local pathophysiology may regain a premenopausal status
- Quality of evidence is "low" or "very low" and RCTs are not available

ABSTRACT

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This study aimed to identify and then synthesize all available data regarding the efficacy of laser therapy for postmenopausal women with genitourinary syndrome of menopause (GSM) with/without urinary incontinence (UI). PubMed, Scopus, Web of Science, Cochrane Library and ClinicalTrials.gov were searched in October 2016. The keywords were "laser genitourinary syndrome of menopause", "laser vulvovaginal atrophy", "laser vaginal atrophy" and "laser women incontinence". Quality of reporting and risk of bias of the included studies were assessed according to STROBE and MINORs checklists, respectively. Quality of the body of evidence was evaluated with the GRADE approach. Fourteen studies involving 542 participants were included in this systematic review and meta-analysis. All GSM symptoms (dryness/dyspareunia/itching/burning/dysuria/urgency/frequency) and UI decreased significantly and consistently in all available publications. The pooled mean differences for the various symptoms were: dryness -5.5(95%CI:-6.7,-4.4;7studies;I^2:0%), dyspareunia -5.6(95%CI:-6.8,-4.5;7 studies;I^2:0%), itching -4(95%CI:-5.7,-2.2;6 studies;I^2:79%), burning -3.9(95%CI:-5.9,-2;6 studies;I^2:87%), dysuria -2.9(95%CI:-5.1,-0.7;4 studies;I^2:90%) and UI -4.9(95%CI:-6.4,-3.4;2 studies;I^2:0%). Because urgency/frequency was assessed by different methodologies the data could not be meta-analyzed. Furthermore, KHQ, UDI-6, MCS12/PCS12, FSFI, overall sexual satisfaction and measurements of the effect of laser therapy on the local pathophysiology improved significantly. In conclusion, laser therapy for postmenopausal women with GSM appears promising. It may reduce symptom severity, improve quality of life of postmenopausal women and restore the vaginal mucosa to premenopausal status. However, the quality of the body of evidence is "low" or "very low" and, thus, evidence-based modification of current clinical practice cannot be suggested.

Keywords: genitourinary syndrome of menopause (GSM), lower urinary tract symptoms (LUTS), incontinence, dyspareunia, Female Sexual Function Index (FSFI), laser therapy

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INTRODUCTION

The urinary and the genital tract systems have the same embryological origin and are both under the influence of estrogens[1]. Decline of estrogens during menopause results in symptoms and clinical signs from both systems, defining the Genitourinary Syndrome of Menopause (GSM)[2]. Depending on type and severity of symptoms, various therapeutic strategies are available[3-7].

Intravaginal laser therapy, a recently introduced treatment modality, has been proposed for the treatment of GSM and/or urinary incontinence (UI)[8-13]. Two laser-technologies, Microablative fractional CO_2-laser (CO_2-laser) (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy) and Non-ablative photothermal Erbium:YAG-laser (Er:YAG-laser) (Fotona Smooth^{TM} XS, Fotona, Ljubljana Slovenia) have been used in postmenopausal women with GSM and/or UI[8-13].

In the current literature 3 reviews regarding the intravaginal use of CO_2 or Er:YAG laser-technologies are available[8-10]. They suggested that laser-therapy may improve the vaginal epithelium and alleviate GSM symptoms of postmenopausal women[8-10]. However, these reviews were not systematic nor meta-analyses and a critical appraisal of the evidence using validated tools was not performed. Furthermore, newer studies have been published that are not included in the above reviews.

The aim of this systematic review was to identify and meta-analyze the available evidence regarding the efficacy of intravaginal laser-therapy in postmenopausal women with symptoms and clinical signs of GSM with/without UI. Specifically, we aimed to systematically summarize-synthesize evidence on objective and subjective measurements of symptoms and clinical signs of postmenopausal women with GSM following laser-therapy compared to those not receiving or

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before receiving one. We also investigated whether the available data may provide evidence-based change in current clinical practice.

METHODS

PubMed, Scopus, Web of Science, Cochrane Library and ClinicalTrials.gov were searched in October 2016. For each database 4 search-strategies were performed. For each search-strategy, one of the following combinations of keywords was used "laser genitourinary syndrome of menopause" or "laser vulvovaginal atrophy" or "laser vaginal atrophy" or "laser women incontinence". No limits were used for either database. Articles full texted, published in peer-reviewed journals, written in English language, using intravaginal-laser in postmenopausal women for the management of GSM with/without UI were eligible to be included in this study. Hand search of the reference lists of the eligible articles was also performed to achieve complete coverage of the literature and limit publication bias. Conference abstracts without full text publication and unpublished studies were excluded. "Grey literature" was not searched.

Data were extracted for the following aspects: First author, year of publication, study design, type of laser-technique, number of participants, baseline characteristics of postmenopausal women, therapeutic protocol, follow-up period, subjective measurements of GSM symptoms (dryness, dyspareunia, itching, burning, dysuria, frequency, urgency, urinary tract infections) and UI, tools of assessing Quality of Life, sexual function, objective measurements of the laser effect on the local pathophysiology, adverse events and drop-outs due to adverse events. Subjective measurements of GSM symptoms and UI defined the primary outcomes. All other outcomes were considered as secondary ones.

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Additionally, all authors independently evaluated the studies design for quality of reporting, risk of bias and quality of evidence. Quality of reporting was assessed using Strengthening the Reporting of Observational studies in Epidemiology (STROBE) checklist[14]. Risk of bias assessment was performed using the methodological index for non-randomized studies (MINORS) checklist[15,16]. A study with score ≤50% of maximum score (8 for uncontrolled before- and after-studies and 12 for controlled ones) was considered belonging in "high risk of bias" category[16]. Quality of the body of evidence was assessed by the GRADE system of rating for the outcomes that could be meta-analyzed[17]. Any discrepancies were resolved by the consensus of all authors. All types of study design, regardless of quality, were considered for this systematic review and meta-analysis. Meta-analysis of each outcome included studies with the same methodology of assessment that provided the relevant data not only p-values.

Heterogeneity between studies was assessed using I^2 statistic[17,18]. Publication bias was intended to be assessed by appropriate tests (i.e funnel-plot), with their known limitations[19,20]. Estimated overall laser effect despite laser-technology [pooled mean difference and 95% confidence intervals (CIs)], was computed (when ≥2 studies were involved) using Review Manager 5.3 and data type of generic inverse variance, using inverse variance as statistical method, random effects model and difference in means as effect measure. Additionally, subgroup-analyses estimating CO_2-laser or Er:YAG-laser efficacy were intended to be performed using the above described methodology.

RESULTS

The systematic process for identification of eligible studies to be included in the current systematic review is presented in Figure 1. Fourteen studies with 542 participants were considered eligible to be included in this review[21,34].

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Main characteristics of the included studies are presented in Table 1. Ten studies used CO_2-laser (SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy), while 4 studies used Er:YAG-laser (Fotona Smooth^{TM} XS, Fotona, Ljubljana Slovenia)[24,27-29]. Twelve studies were prospective uncontrolled before- and after-studies (10 CO_2-laser and 2 Er:YAG-laser)[21-23,25-27,29-34]. Two studies were prospective controlled before- and after-studies, using estriol as control-group (both used Er:YAG-laser)[24-28].

In all studies treatment protocol involved 3 laser-therapies. Assessment of outcomes was performed before the initiation of laser-therapy and after the last laser-therapy. The time-period between last laser-therapy and assessment of outcomes was defined as follow-up period. In all but 2 studies dyspareunia and dryness were assessed using the Visual Analogue Scale (VAS) 0-10[22,23,25-33], while in 1 study VAS 0-5[21] and in 1 VAS 0-3[24]. Hence, these studies[21,24] could not contribute in the meta-analysis. In all studies itching, burning and dysuria were assessed by VAS 0-10. Frequency and urgency were assessed following different methodologies such as micturition diaries[26] and questionnaires (Overactive Bladder-Questionnaire short form (OAB-Q SF)[26], the International Consultation on Incontinence Questionnaires (ICIQ-FLUTS) (filling domain) and the Urinary Distress Inventory-6 (UDI-6)[22]. Hence, they could not be meta-analyzed. UI was assessed by micturition dairies[26], UDI-6[22] and ICIQ-Urinary Incontinence Short Form (ICIQ-UI SF)[22,27,28]. None of the studies investigated the efficacy of laser therapy in patients with a history of UTIs or rUTIs.

Outcome data of included studies are presented in Table 1. The “high risk of bias” category of the quality assessment did not apply to any of the studies. Tests for publication bias although intended to be assessed, were not assessed following the recommendations on testing funnel-plot asymmetry by Cochrane[19]. The quality of the body of evidence is reported in Supplementary Table 1.

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1.1 Primary outcomes

All primary outcomes are presented in Table 2. The forest plots of the meta-analyses at 1-month follow-up are presented in Figure 2. All primary outcomes decreased significantly in all relevant studies. Overall UI decreased significantly at 1-month follow-up and the result maintained up to 6-months follow-up[22,26-28]. Quality of the body of evidence rated "low" for dryness, dyspareunia and UI. Quality of evidence rated "very low" for itching, burning and dysuria (Supplementary Table 1).

In subgroup-analysis of CO_2-laser the pooled mean difference of dryness and dyspareunia was -5.5 (95%CI:-6.6, -4.4;p<0.00001, I^2:0%;n=255) and -5.5 (95%CI:-6.6, -4.4;p<0.00001; I^2:0%;n=229), respectively[22,26,30-33]. At 3-months follow-up (VAS 0-10) mean difference of dryness and dyspareunia was -6.1 and -5.4, respectively (n=27)[25].

Subgroup-analysis of Er:YAG-laser group was not performed due to lack of data of 2[27,29] out of 3 relevant studies. However, in all studies the significant improvement of dryness and dyspareunia maintained up to 18-months follow-up. Estriol-group maintained the significant improvement up to 6-months after last application with a subsequent significant worsening of symptoms at 18-months post-estriol treatment.

1.2 Secondary outcomes

Female Sexual Function Index (FSFI) and overall sexual satisfaction of participants (as evaluated by a VAS 0-10), Vaginal Health Index (VHIS) and Vaginal Maturation Value (VMV) increased significantly in all relevant studies (Table 3, Figure 3). At 1-month follow-up 85%-98% of participants that were not sexually active at baseline resumed their sexual activity[22,31]. Quality of

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the body of evidence rated “low” for FSFI, overall sexual satisfaction and VHIS and “very low” for MCS12, PCS12 and VMV (Supplementary Table 1).

In subgroup-analysis of the CO_2-laser effect on VHIS the pooled mean difference was 10.8 (95%CI:8.7-12.9;p<0.00001, I^2:0%;n=231)[21,22,26,30,32,33]. VHIS increased significantly in breast-cancer survivors irrespectively of receiving or not adjuvant therapy (aromatase inhibitors or tamoxifen) (n=50)[30]. Furthermore, VMV increased significantly by Er:YAG-laser and estriol application but the mean VMV values were higher following laser-therapy[28]. At 1 month-follow-up Er:YAG-laser and estriol improved VHIS similarly, but at 3-months follow-up Er:YAG-laser was superior to estriol as regards VHIS[28].

King’s Health Questionnaire (KHQ) total score decreased significantly from mean 235.9 before laser-therapy to 114.1 at 1-month follow-up[22]. A change from baseline of at least 5 points on King's Health Questionnaire domains (minimal clinically important difference -MCID[35], was exceeded in all KHQ domains of all participants (n=35)[22]. Additionally, UDI-6 scores decreased significantly (total score, Q1, Q2, Q3 and Q4)[22].

Histopathological changes of vaginal mucosa were observed up to 12-months follow-up (2 studies, n=11)[24,34]. Specifically, there was increase of the vaginal epithelium thickness as well as improvement in vascularization and angiogenesis penetrating the new papillary formation. Fibroblasts number and synthesis of fibrilar components of extracellular matrix was also increased. Additionally, the histopathological study evaluating the CO_2-laser effect reported high levels of glycogen, stored in large epithelial cells and augmented exfoliation of superficial epithelial cells.

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A microbiological study using Gram-stain as a method of assessment, reported significant increase of vaginal epithelial cells (n=53)[23]. Vaginal microenvironment changed, with significant improvement of normal vaginal flora, increase of lactobacilli and decrease of *E. coli* and *Mobiluncus*. Specifically, prevalence of normal flora and lactobacilli rose from 45% to 90% and from 30% to 79%, respectively. In 2 studies (n=78), vaginal fluid's pH decreased significantly from mean 5.5 to 4.7 and from 5.0 to 4.1 (before, 1- and 3-months follow-up, respectively)[23,24]. However, in 1 study (n=27) a trend of pH increase at 3-months follow-up was observed[25].

The Patients Global Impression of Improvement (PGI-I) rated "very much better/much better" at 1- and 3-months follow-up in 90% and 73% of participants, respectively (n=80)[22,25]. At 1-month follow-up, 5-point Likert scale rated "very satisfied/satisfied" in 61% to 100% of participants (n=188)[21,25,30,32,33]. Moreover, 76% and 52% of breast cancer survivors were 'Very satisfied/satisfied" at 1- and 11-months' follow-up, respectively (n=50)[21]. Additionally, 85%-95% stated that laser procedure was "excellent/excellent-good" (n=108), while for 84% of participants estriol procedure was "excellent-good" (n=19)[27,28].

Six studies (n=230) reported that no adverse events were noted. Adverse events included pain during probe insertion[21], discomfort related to first application[27], mild irritation of the introitus starting immediately after laser-therapy and resolving spontaneously in about 2 hours[22,23], mild or moderate pain lasting 2-3 days[25,28], minor bleeding or spotting[24,25], sensation of warmth and slight edema[24]. Two out of the 542 participants discontinued therapy due to discomfort related to first application/burning sensation that started 36 hours post-therapy and lasted for 2 days. Both women received Er:YAG-laser therapy.

DISCUSSION

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Intravaginal laser-therapy, is a recently proposed treatment modality for the management of GSM with/without UI. This systematic review and meta-analysis assessed the efficacy and safety of laser-therapy in postmenopausal women with GSM/UI and evaluated also the quality of evidence. Decrease of GSM symptoms and UI severity, has been indicated by current literature consistently. The described symptoms' relief or amelioration appeared to be in accordance with the reported histopathological changes of vaginal mucosa, restoration of the vaginal flora and increase of VHIS and VMV. Quality of life, sexual function and overall satisfaction with sexual life of women receiving laser-therapy seemed to improve significantly not only in statistically but also in clinically meaningful levels. The implied beneficial effects of laser-therapy were ratified by the PGI-I and 5-point Likert scale with the clear majority of participants stating "very much better/better" and "very satisfied/satisfied" following 3 laser-sessions, respectively. However, no Randomized Controlled Trials were retrieved. The preponderance of studies was uncontrolled before- and after-studies and quality of the body of evidence rated "low" or "very low".

Dryness and dyspareunia are considered the most common and bothersome GSM symptoms[36-38]. They tend to worsen in time with their initial appearance to be estimated at about 1-year after the last menstrual period[37]. Low-dose vaginal estrogens are the gold standard of local therapy and the available quality of evidence, comparing estrogens to placebo, has been rated "moderate"[39]. However, for breast-cancer survivors' estrogens should be retained as second line therapy[40]. In this review although the quality of evidence was low, dryness and dyspareunia consistently improved in all studies, regardless of the laser-technique. Controlled studies comparing Er:YAG-laser therapy to estriol found similar effect in both groups of participants with laser-group maintaining the beneficial effect for longer period[24,28]. Nevertheless, no safe conclusions can be deducted as in one of these studies estriol cream was applied prior to laser-therapy and different range of VAS for the assessment of symptoms' severity was used. A future evaluation of combined therapy using low-

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dose estriol and Er:YAG-laser could be of great interest. In a study including postmenopausal breast-cancer survivors' dyspareunia decreased significantly in similar manner to the other studies included in this review[21]. These results were also in accordance to a study evaluating CO_2-laser therapy in breast cancer survivors of all ages[41].

Itching, burning and dysuria decreased significantly following CO_2-laser therapy but quality of evidence rated "very low" for all of them. Heterogeneity of laser effect between the various studies could possibly be explained by the criteria of participants' inclusion in the analysis of each study. In 1 study[22], all participants were included in the analysis regardless of presence or not of these symptoms prior to laser-therapy. In other studies, only the participants with the above mentioned symptoms at baseline were analyzed[26,30-33]. This discrepancy, indicates that significant improvement of itching, burning and dysuria following the CO_2-laser is apparent, even when underestimated. Likewise, amelioration of these symptoms was observed at 3-months' follow-up[25].

Moreover, symptoms from the lower genital tract system are strongly interrelated to LUTS[3], while vaginal dryness was found to be associated with greater prevalence of UI[42]. Vaginal estrogens could be proposed for postmenopausal women with UI, especially when GSM symptoms coexist[43]. Quality of evidence for vaginal-estrogens efficacy compared to placebo is of "low-quality" for urgency, "very low-quality" for frequency/nocturia, "low-quality" for SUI and "moderate-quality" for UUI[39]. Quality of evidence for laser-therapy rated "very low" for dysuria and "low" for UI overall, while heterogeneity of urgency/frequency assessment, did not allow meta-analysis of the data or defining the quality of evidence. Available studies did not provide data of objective UI measures. However, the results seemed to be promising with all relevant studies reporting significant improvement of all LUTS regardless of method of assessment. Mean difference

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of ICIQ-UI SF of women with SUI was -6.5 and maintained up to 6-months follow-up[28], while meta-analysis of ICIQ-UI SF for women having UI was -4.89 (95% CI -6.38, -3.41). Threshold of MCID of ICIQ-UI SF for women with SUI has been reported to be (-5)[44], (-4)[44] or (-2.52)[45] depending on follow-up period and type of treatment. Although the results of our study concerned follow-up period up to 6 months, it would be of great interest to see whether this clinically important effect will be confirmed by large controlled trials with a long-term follow-up.

Furthermore, the validated questionnaires for LUTs (KHQ and UDI-6), seemed having a statistically and clinically meaningful improvement but the number of evaluated participants was small[22]. All scores of the KHQ domains exceeded the 5-point threshold of MCID, implying further a promising impact of laser-therapy in quality of life of postmenopausal women with UI and possibly a positive effect in UI pathophysiology. Likewise, UDI-6 indicated an analogue influence[22]. Nevertheless, the evidence is scarce and further research is warranted.

GSM and UI has a negative effect on sexual function[46,47]. Improvement of FSFI was consistent in all studies but the quality of evidence “low”. An FSFI threshold of 26.55 is considered to define sexual dysfunction[48]. The highest the score the better is sexual function. Although FSFI scores were higher after 3 laser-therapies indicating better sexual function following laser-therapy, the clinically meaningful threshold of sexual dysfunction was not achieved in all studies. However, sexual function is impaired by many psychological and social factors[49] and is encouraging that almost all women not having sexual activity due to symptoms’ severity, after the laser-therapy resumed their sexual life with a clinically meaningful improvement of their overall satisfaction with sexual life.

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Histopathological, microbiological, cytological and macroscopical findings indicated the possible effect of laser-therapy on the vaginal mucosa and microenvironment[21-30,32-34]. Although the quality of evidence of VHIS increase was low, there was consistency of results, with the threshold of 15 (which defines vaginal atrophy) exceeded in all studies. Moreover, lactobacilli and normal vaginal flora appeared to increase, while pathogens i.e *E. coli* and *Mobiluncus* appeared to decrease at 1-month follow-up. Vaginal colonization with pathogens is considered the predisposing factor for presence and recurrence of UTIs[50]. Hence, indirectly, assumptions of laser's preventive ability for UTIs may be made based on restoration of local pathophysiology after 3 laser-therapies. However, PCR studies identifying the *Lactobacillus* species and the whole vaginal microbiota are needed for determination of the possible vaginal protection following laser-therapy.

This study has several limitations. Includes only full text articles, published in English language in peer-reviewed journals and although several databases were searched incomplete retrieval of identified studies cannot be overruled. Moreover, publication bias could not be assessed. The clear majority of identified studies were uncontrolled before- and after-studies. Hence, possible placebo effect of the treatment cannot be overruled. Moreover, the 2 controlled studies using estriol as control group, could not be meta-analyzed. Finally, samples used for histopathological evaluation were narrow and statistical analysis was not presented.

In conclusion, laser-therapy seems a promising and safe non-pharmaceutical therapeutic option for GSM in both clinical and pathophysiological aspect. However, quality of the body of evidence is "low" or "very low", the possible placebo effect of the treatment has not yet been ruled out or estimated, while there is scarce data regarding the use of laser in women with GSM and UI. Well-designed controlled studies with standardized laser settings and therapeutic protocols, long duration of follow-up, consistent outcome evaluations, comparing laser-therapy to placebo or other treatment

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modalities and/or evaluating the pathway and mechanism of action on the vaginal mucosa are essential to be performed for safe conclusions to be derived. Currently, evidence-based change in clinical practice for the management of GSM with/without UI, cannot be proposed.

Contributors

All five authors contributed to the conception of the review, the acquisition, analysis and interpretation of the data, and the drafting of the manuscript.
All authors saw and approved the final version of the manuscript.

Conflict of interest

Stefano Salvatore has had financial relations (expert testimonies and lectures) with DEKA Laser. The other authors report no potential conflict of interest.

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This article has undergone peer review.

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Table 1. Main characteristics reported in the studies included in the systematic review and meta-analysis.

First Author [Ref]	Study design*	Type of laser*	No of patients	Baseline characteristics of postmenopausal women*	Therapeutic protocol*	Follow-up*	STROBE*	MINORS*
Pieralli[21]	PUBA	CO_2-laser	50	Mean age 53.3 (range41-66), current or previous breast cancer, dyspareunia	3 laser-therapies (1/m)	Mean 11m†	20/3/11	13/16
Pitsouni[22]	PUBA	CO_2- laser	53	Mean age 57.2±5.4, moderate to severe symptoms of GSM	3 laser-therapies (1/m)	1m	27/1/6	14/16
Athanasiou[23]	PUBA	CO_2 –laser	53	Mean age 57.2±5.4, ≥1 moderate to severe symptoms of GSM, vaginal pH>4.5, superficial epithelial cells<5%	3 laser-therapies (1/m)	1m	25/4/5	15/16
Gaspar[24]†	PCBA	Erbium:YAG laser vs estriol	50 25 vs 25	Non-smokers, estradiol level≤20pg/ml, >1symptoms: dyspareunia, vaginal dryness, vaginal burning or irritation, chronic leukorrhea	0.5mg estriol ovules for 2wks (3/wk) and then 3 laser-therapies in 8 wks	18m	18/4/12	20/24
Sokol[25]	PUBA	CO_2-laser	27	Mean age 58.6±8.8, healthy, with bothersome GSM symptoms (pain, burning, itching, dryness, dyspareunia, dysuria), non-smokers	3 laser-therapies (1/6 wks)	3m	20/6/8	11/16
Perino[26]	PUBA	CO_2- laser	30	Median age 56, ≥1symptoms of GSM(itching, burning, reduced lubrication, superficial and/or severe dyspareunia) and symptoms of OAB ≥3m (≥8 times micturition/24h, ≥3 episodes of urgency (grade 3 or 4) with or without incontinence in 3-day voiding diary)	3 laser-therapies (1/m)	1m	20/4/10	13/16
Gambacianni[27]	PUBA	Erbium:YAG laser	65	Mean age 62.9±8.1, presence of a GSM (vaginal dryness or dyspareunia), FSH>40, Estradiol<25	3 laser-therapies (1/m)	1m	22/6/6	13/16
Gambacianni [28]‡	PCBA	Erbium:YAG laser vs estriol	62 43 vs 19	Mean age 60.9±8.1 and 63±4.5, GSM symptoms (vaginal dryness and dyspareunia), SUI, FSH>40, estradiol<25	3 laser-therapies (1/m)	6m	26/2/6	17/24
Gambacianni[29]	PUBA	Erbium:YAG laser	13	Mean age 55.7±7.7, successful treatment for breast cancer, severe GSM symptoms (vaginal dryness and dyspareunia), FSH>40, estradiol<25	3 laser-therapies (1/m)	3m	17/5/12	13/16
Perino[30]	PUBA	CO2-laser	48	Median age 56, 1≥ GSM symptoms (itching, burning, reduced lubrication, superficial and/or severe dyspareunia)	3 laser-therapies (1/m)	1m	16/6/12	13/16
Salvatore[31]	PUBA	CO2-laser	75	Mean age 60.6±6.2, moderate/severe vaginal dryness and/or dyspareunia	3 laser-therapies (1/m)	1m	24/1/9	14/16
Salvatore[32]	PUBA	CO2-laser	49	Mean age 59.6±5.8, moderate/severe vaginal dryness and/or dyspareunia	3 laser-therapies (1/m)	1m	19/3/12	13/16
Salvatore[33]	PUBA	CO_2-laser	15	Mean age 57.3±3.0, sexually active with dyspareunia related to GSM	3 laser-therapies (1/m)	1m	19/3/12	13/16
Zerbinati[34]§	PUBA	CO_2-laser	5	Mean age 57±1.7, severe GSM symptoms (vaginal dryness, burning, itching, dysuria, dyspareunia).	3 laser-therapies (1/m)	2m	15/4/14	11/16

*PUBA: Prospective Uncontrolled Before (the initiation of the laser-therapy) and After (the last laser-therapy). PCBA: Prospective Controlled Before (the initiation of the laser-therapy) and After (the last laser-therapy); CO_2-laser: Microablative Fractional CO_2-laser ((SmartXide^2 V^{2}LR, Monalisa Touch, DEKA, Florence, Italy), Erbium:YAG-laser: Non-ablative thermal Erbium:YAG-laser ((Fotona Smooth™ XS, Fotona, Ljubljana Slovenia); GSM: Genitourinary Syndrome of Menopause. SUI: Stress Urinary Incontinence, OAB:Overactive Bladder; m: months, wks:weeks;. Follow-up: the time following the last laser-therapy in which outcomes assessment was performed; STROBE checklist was used for the Quality of Reporting of studies. Each item of the STROBE checklist could take one of the following values: Yes/No/Not applicable. The numbers presented are the sum for each of these values. MINORS checklist was used for the assessment of the Risk of Bias of the included studies. Scores of 0, 1 or 2 are applied when the items are not reported, inadequately reported or adequately reported, respectively. The numbers presented are the sum of these scores.

#Range 3-25m

‡ Women included in the Estriol group followed the protocol: 8 wks 0.5mg estriol ovules (1/d for the first wk, 3/wk in 2-4wks, 2/wk in wks 4-8

§This study presented the histopathological data of 5 women participating in the study of Salvatore et al [32].

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Table 2. Primary outcomes of the studies included in this systematic review and meta-analysis.

First Author[Ref]	Dryness*	Dyspareunia*	Itching*	Burning*	Dysuria*	Urgency*	Frequency*		Urinary Incontinence*
Pieralli[21]	NA	B:5(1-5) A:3 (1-5)(1m)	NA	NA	NA	NA	NA		NA
Pitsouni[22]	B:6.1±3.1 A:1.7±1.9 (1m)	B: 7.7±2.5 A: 2.3±2.2 (1m)	B:1.7±3.2 A: 0.3±1.2 (1m)	B:1.3±2.9 A:0.3±0.9 (1m)	B:0.9±1.7 A: 0.3±0.7 (1m)	B:1.2±1.0 A: 0.3±0.5 (1m)	Day-freq B:0.9±0.9 A:0.6±0.7 (1m)	Nocturia B:1.3±1.0 A:0.6±0.7 (1m)	UI (ICIQ-UI SF) B: 8.1±5.6 A: 3.4±4.3(1m) SUI: B:1.6±1.5/A:0.5±0.9(1m) UUI: B:1.6±1.7/A: 0.7±1.3(1m)
Gaspar[24]§	B: 2.2±0.6 A: 1.1±0.8(1m) 1.0±0.7(12m) 1.5±0.7(18m)	B: 2.4±0.7 A: 1.5±0.8(1m) 0.9±0.9(12m) 1.6±0.7(18m)	NA	NA	NA	NA	NA		NA
Sokol[25]	Improvement 6.1±2.7(3m)	Improvement 5.4±2.9(3m)	Improvement 1.3±1.9(3m)	Improvement 1.4±2.9(3m)	Improvement 1.0±2.4(3m)	NA	NA		NA
Perino[26]	B: 8(3) A: 2(1.3)(1m)	B: 9.5(1.3) A: 2(1.3)(1m)	B: 8(2) A: 1(1)(1m)	B: 8(2.3) A: 1(1)(1m)	NA	B:3(0)† A:0(1)(1m) B:18.5±4.3‡ A:8±2.3(1m)	B: 10(2.5) A: 6(2)(1m)		UUI (micturition dairy) B: 3(1) A: 1(1.5)(1m)
Gambacianni [27]	Decrease (1m)	Decrease (1m)	NA	NA	NA	NA	NA		SUI (ICIQ-UI SF) decrease (1m)
Gambacianni [28]‖	B: 8.3±1.3 A: 2.9±0.6(1m) 3.5±0.9(6m)	B: 8.2±1.3 A: 2.8±1(1m) 3.5±1.1(6m)	NA	NA	NA	NA	NA		SUI (ICIQ-UI SF) B: 12±1.8 A: 5.5±2.6(1m) 5±2.6(6m)
Gambacianni [29]	Decrease (1m and 3m)	Decrease (1m and 3m)	NA	NA	NA	NA	NA		NA
Perino[30]	B:8(2) A: 2(1)(1m)	B:8(2) A: 3(1)(1m)	B:6(1.8) A: 2(0.8)(1m)	B:6(2) A: 2(1)(1m)	NA	NA	NA		NA
Salvatore[31]	B: 8.4±2.0 A:2.8±1.8(1m)	B: 8.4±2.4 A:2.8±2.1(1m)	B: 6.4±2.1 A:2.1±2(1m)	B: 6.2±2.7 A:2.2±2.8(1m)	B: 5.7±2.8 A:2.6±1.9(1m)	NA	NA		NA
Salvatore[32]	B: 8.3±2.1 A:2.7±1.9(1m)	B: 8.1±2.8 A:3.3±2.3(1m)	B: 6.1±3 A:1.5±1.7(1m)	B: 6.4±2.7 A:2.9±2.4(1m)	B: 5±2.4 A:1.1±1.1(1m)	NA	NA		NA
Salvatore[33]	B: 7.2±1.1 A:1.7±0.9(1m)	B: 8.7±1 A:2.2±1(1m)	B: 5.6±1.3 A:1.6±0.7(1m)	B: 6.9±2.7 A:1.5±1.9(1m)	B: 5.1±0.9 A:0.8±1(1m)	NA	NA		NA

* All p-values for all outcomes at all follow-up periods were statistically significant ±p<0.005);.B: Before ±the initiation of laser-therapy, A: After ±the last laser-therapy, NA: Not applicable, UI: Urinary Incontinence, SUI: Stress Urinary Incontinence, UUI: Urgency Urinary Incontinence; . In all but 3 studies data were reported as mean ±standard deviation. In 1 study [21] data were reported as median and range (range is presented in the parenthesis) and in 2 studies [26, 30] data were reported as median and interquartile (interquartile is presented in the parentheses). The time of outcome assessment after the last laser-therapy is presented in the parenthesis (e.g 1m, 12m) (m: months); In all but 2 studies symptoms severity was assessed using Visual Analogue Scale ±VAS) 0-10, while in 1 study VAS 0-5 [21]and in 1 study VAS 0-3 [24]; In one study the calculation of day frequency/nocturia, UI, UUI/SUI, was assessed using ICIQ-FLUTS ±filling domain), ICIQ-UI SF and UDI-6, respectively[22]. In one study the calculation of frequency, urge episodes were performed by micturition dairy and Overactive Bladder-questionnaire ±OAB-q) [26]. In 2 studies the calculation of SUI was performed by the ICIQ-SF [27,28].

† Number of urge episodes was assessed using micturition dairy.

‡ Urgency was assessed using OAB-q.

§ The data presented concern the laser-therapy. Estriol-group: Dryness decreased significantly up to 6m [from 2.3 ±0.5 to 1.3 ±0.9(1m) and 1.7±0.8 (6m)]. At 12 and 18m dryness was 2.4±0.7). Dyspareunia decreased significantly up to 6m [from 2.3 to 1.8±0.8(1m) and 1.9±0.8(6m). At 12 and 18m dyspareunia was 2.2±0.7 and 2.4±0.7, respectively.

‖ The data presented concern the laser-therap. Dryness: Estriol group had similar decrease during the treatment period and no significant differences between groups. After the end of treatment small but significant increase. At 6m the estriol group was significantly different from laser-therapy group. Dyspareunia: Similar decrease during the treatment period and no significant differences between groups. After the end of treatment small but significant increase. At 6m the estriol group was significantly different from laser-therapy group.

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Table 3. Secondary outcomes of the studies included in this systematic review and meta-analysis.

First Author[Ref]	KHQ*	UDI-6*	FSFI*	MCS12*	PCS12*	pH*†	VHIS*	VMV*
Pieralli[21]	NA	NA	NA	NA	NA	NA	B:8.9±1.7 A:21.6±1.6(1m)	NA
Pitsouni[22]	B:235.9±226.1 A:114.1±165.8(1m)	B: 30.2±21.4 A: 11.9±16.6(1m)	B:13.7±8.1 A: 25.9±4.6(1m)	NA	B:0.9±1.7 A: 0.3±0.7(1m)	B:2.7±1.1 A: 4.2±1.7(1m)	B:8.4±2.5 A:20.1±3(1m)	B: 11.7±15.6 A: 44.2±13.7(1m)
Athanasiou[23]	NA	NA	NA	NA	NA	B:5.5±0.8 A: 4.7±0.5(1m)	NA	NA
Gaspar[24]‡	NA	NA	NA	NA	NA	B:5±0.4 A: 4.1±0.4(3m) 4.4±0.6(6m)	NA	B: 20.8±5.4 A: 33.3±13.3(1m) 52.2±8.5(12m)
Sokol[25]	NA	NA	Improvement 8.8±7.3(3m)	NA	NA	B:5.5±0.7 A: 5.7±1.4(3m)	B:14.4±2.9 A:21.4±2.9(3m)	NA
Perino[26]	NA	NA	NA	NA	NA	NA	B:11±3 A:22±3.3(1m)	NA
Gambacianni [27]	NA	NA	NA	NA	NA	NA	Increase (1m)	NA
Gambacianni [28]§	NA	NA	NA	NA	NA	NA	B:10.6±3.6 A:20±1.4(1m) 19.0±1.4(6m)	NA
Gambacianni [29]	NA	NA	NA	NA	NA	NA	Increase (1m and 3m)	NA
Perino[30]	NA	NA	NA	NA	NA	NA	B:10.5±3 A:21.5±2(1m)	NA
Salvatore[31]	NA	NA	B: 14.8±7.7 A:27.2±5.6(1m)	B: 43.2±8.3 A:46.1±7.6(1m)	B: 48.8±6.4 A:50.7±6.5(1m)	NA	NA	NA
Salvatore[32]	NA	NA	NA	B: 42.6±10.6 A:47±6.9(1m)	B: 44.6±11 A:50±5.9(1m)	NA	B:13.1±2.5 A:23.1±1.9(1m)	NA
Salvatore[33]	NA	NA	B: 12.2±1 A:27.3±0.9(1m)	B: 41.9±11.7 A:50.7±1.1(1m)	B: 43.8±10.9 A:55.7±0.3(1m)	NA	B:12.9±3 A:22.1±2.3(1m)	NA

* In all but 1 study all outcomes at all follow-up periods improved statistically significant ±p<0.005);.In 1 study pH did not decrease [25]; VHIS: Vaginal Health Index Score ± VHIS is calculated by adding the scores of the 5 components: Elasticity, fluid volume, pH, epithelial integrity and moisture. Each component could receive a score from 1 ±poorest) to 5 ±best). The sum of the 5 components could receive an upper bound score of 25 and lower bound of 5. A Score of ≤15 defined the presence of vaginal atrophy), VMV: Vaginal Maturation Value ±±Parabasal epithelial cells, Intermediate epithelial cells and superficial epithelial cells were quantified as percentages in the vaginal smear. VMV was calculated using the formula ±±1x%superficial) +±0.5x%intermediate) +±0x%parabasal)). B: Before ±the initiation of laser-therapy, A: After ±the last laser-therapy, NA:Not Applicable; In all studies data were reported as mean ±standard deviation. The time of outcome assessment after the last laser-therapy is presented in the parenthesis (e.g 1m, 6m) (m: months).

† In all studies pH was evaluated by pH indicators strips. In all but 1 study the pH decrease was reported as evaluated directly by the pH indicators strips. In 1 study the improvement of pH was reported as scored in the VHIS [22].

‡ The data presented concern the laser-therapy. Estriol-group: pH decreased significantly up to 3m [from 4.9 ±0.6 to 4.5 ±0.7 (1m) and 4.5±0.7 (3m)]. At 6m pH was 4.9±0.6). VMV increased significantly up to 6m [from 22.5±7.1 to 26.8±7.1 (1m) and 32.6±13.9 (3m). At 6m VMV was 24.7±13.2 (Non Significant) and at 12m was 25±6.37 (Statistically Significant).

§ The data presented concern the laser-therapy. Estriol-group: VHIS increase statistically significant from 11.2±2.8 to 20.1±3.1 (1m), 15.3±1.5 (3m) and 16.2±1.7 (6m).

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Figure legends

Fig. 1. Flow diagram of the identification process of the studies eligible to be included in this systematic review and meta-analysis**.**

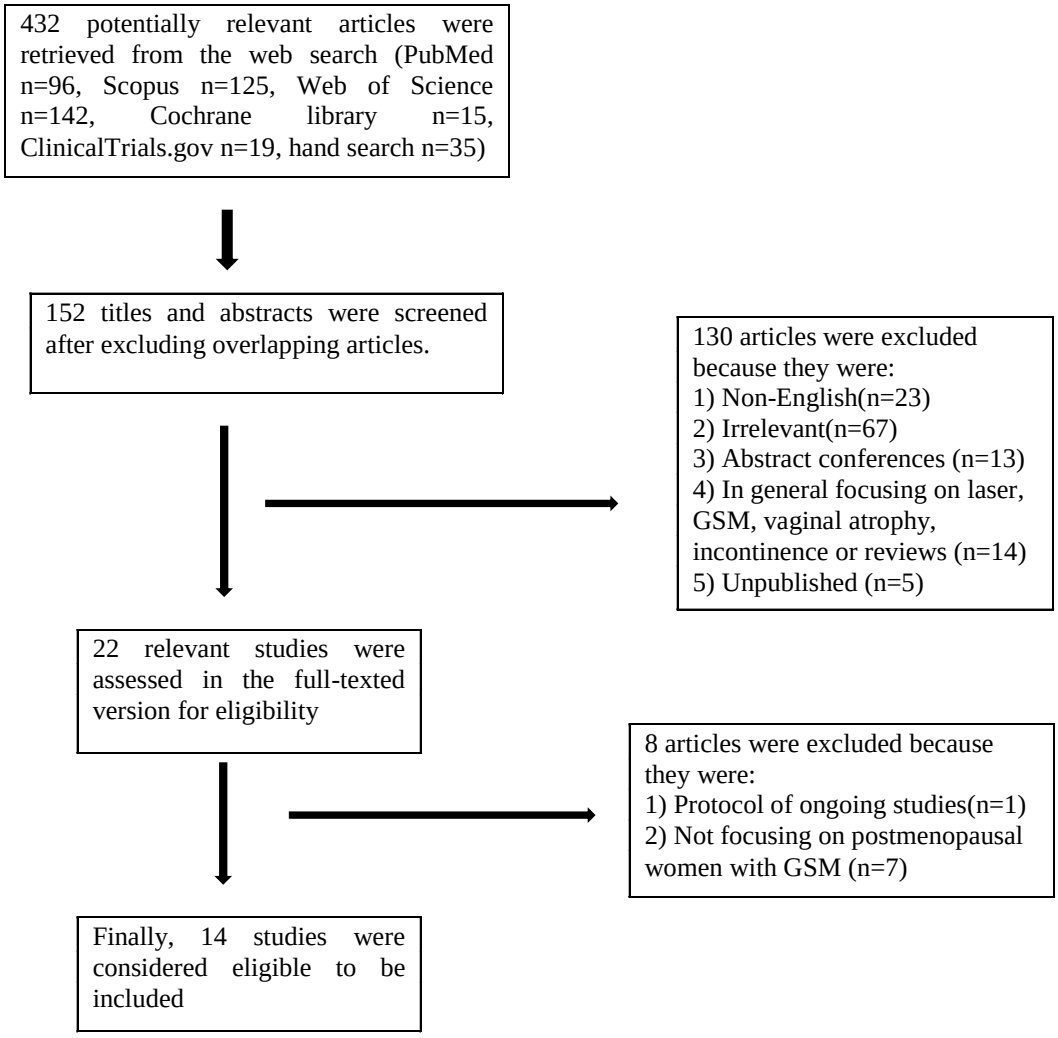

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Fig. 2. Forest plots of mean differences between mean values of before the initiation of laser-therapy and 1-month after the last laser-therapy (1-month follow-up) for the primary outcomes: Dryness n=298, Dyspareunia n=270, Itching n=272, Burning n=281, Dysuria n=185 (assessed by Visual Analogue Scale 0-10) and Urinary Incontinence (n=54) (assessed by the International Consultation on Incontinence Questionnaires-Urinary Incontinence Short Form)

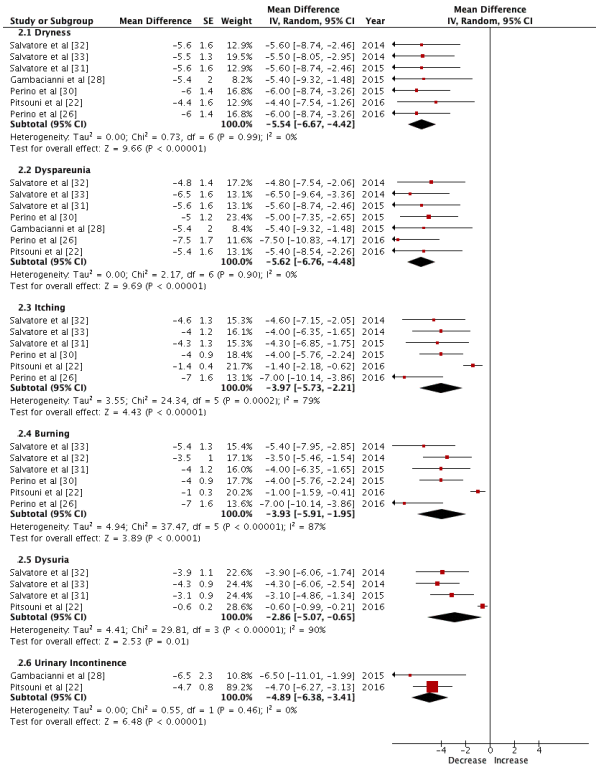

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Fig. 3. Forest plots of mean differences between mean values of before the initiation of laser-therapy and 1-month after the last laser-therapy (1-month follow-up) for the secondary outcomes: FSFI: Female Sexual Function Index n=143, MCS12: Mental Component Summary-12 n=139, PCS12: Physical Component Summary-12 n=139), VHIS: Vaginal Health Index Score n=274 (above 15 is considered the threshold of non-atrophic values, VMV: Vaginal Maturation Value n=78 [is calculated by (1x%superficial)+(0.5x%intermediate)+(0x%parabasal)]