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The effect of fractional microablative carbon dioxide laser therapy on vaginal atrophy in menopause women: A review

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Abstract
Vaginal atrophy is one of Genitourinary Syndrome of menopause. This condition occurs due to decreased estrogen levels during menopause. Epidemiological data obtained from several countries estimated that up to 50% of post-menopausal women experience symptoms of vaginal atrophy. This paper aims to conduct a systematic review, and this meta-analysis is to analyze the effect of micro ablative fractional CO2 laser therapy on vaginal atrophy in postmenopausal women. Data was collected online from Pubmed-MEDLINE, Scopus, EBSCO, Cambridge Core, ProQuest, Springer Link, Cochrane Library, and ClinicalTrials.gov databases. Tracking will be carried out in May-July 2023. The population of this study is the result of clinical trial research on the effect of microablative fractional CO2 laser therapy on vaginal atrophy in postmenopausal women. Meta-analysis showed that the VHI score was higher after therapy in the group that received micro ablative fractional CO2 laser compared to the group that received sham laser for vaginal atrophy in menopausal women. VAS scores were lower after administration in the group that received microablative fractional CO2 laser compared to the group that received sham laser for vaginal atrophy in menopausal women. The incidence of side effects in the CO2 laser group did not differ from the sham laser group. This study recommends an RCT study was conducted comparing micro ablative fractional CO2 laser compared to other energy-based devices such as erbium laser and radiofrequency on vaginal atrophy in postmenopausal women so that it can be used as a basis for further systematic review research and meta-analysis.

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Keywords: Genitourinary Syndrome, Menopause, Meta-analysis, Microablative, Vaginal atrophy

1. Introduction

Vaginal atrophy is one component of Genitourinary Syndrome of menopause. This condition occurs due to decreased estrogen levels during menopause [1]. Epidemiological data obtained from several countries, it is estimated that up to 50% of post-menopausal women experience symptoms of vaginal atrophy [2]. Women will live a third of the time as life expectancy increases to over 80 years. Life after menopause [3]. The course of vaginal atrophy is chronic progressive hence symptoms will worsen over time if not treated [4]. Based on these two things, if women with postmenopausal vaginal atrophy do not receive appropriate therapy, it will have a significant negative impact. This affects the quality of life and sexual function of both sufferers and their partners [3, 4]. However, unfortunately, only 25% of women with symptoms of vaginal atrophy seek medical help. This is due to feelings of shame and considering menopause to be a natural process part of life [4, 5].

The diagnosis of vaginal atrophy is made based on the history and physical examination [3]. Vaginal dryness is the most frequently reported symptom. Other symptoms include dyspareunia, itching, burning, pain and recurrent vaginal infections [1]. On physical examination, the vagina will show petechiae, the fragility of the vaginal walls, decreased elasticity, and loss of rugosity. A

supporting examination that is easy to carry out is a vaginal pH examination. [3] Histopathological examination can be considered if the clinical manifestations are atypical and do not improve after therapy or research interests [6].

The assessment parameters are generally only used in clinical trials [6, 7].

Vaginal Health Index (VHI) is a parameter commonly used to assess vaginal health. There are five assessment components, including vaginal elasticity, secretion fluid volume and consistency, vaginal pH, vaginal mucosal epithelium, and vaginal moisture. The total VHI score ranges from 5 to 25, with a score limit of less than 15, indicating the presence of vaginal atrophy [8, 9]. A visual Analogue Scale (VAS) is used to evaluate subjective symptoms felt by sufferers related to vaginal atrophy, with a value of 0 as the lowest value and a value of 10 as the highest score [9].

The main aim of treating vaginal atrophy is to ensure that the function of the vaginal epithelial tissue returns to its pre-menopausal state, reducing complaints and symptoms; hence sufferer's quality of life can improve, especially sexual function [10]. The choice of therapy is based on the severity of the symptoms and the sufferer's comfort [7, 11].

According to the North American Menopause Society, first-line therapy for vaginal atrophy is non-hormonal therapy. Non-hormonal therapy includes intravaginal lubricants and moisturizers and continuing to have regular sexual intercourse. Lubricants are water, silicone, or oil-based and are applied to the external genitals before sexual intercourse, thereby relieving discomfort during intercourse [12]. Vaginal moisturizers offer greater genital hydration, and according to randomized controlled clinical trials, acidic vaginal gels Hyaluronate effectively improves clinical disorders and can be considered an excellent alternative to estrogen-based treatments in alleviating symptoms of vaginal dryness. However, although lubricants and moisturizers can relieve symptoms, the improvement is often temporary, and repetition is required, which affects patient compliance. Lubricants and moisturizers are also known to be unable to restore the urogenital tract to the state it was in before menopause, but only to increase comfort during sexual intercourse [13].

In cases that do not respond to non-hormonal therapy, hormonal therapy can be given. Systemic administration of Hormonal Replacement Therapy (HRT) is effective in reducing complaints and symptoms related to hypoestrogenism (not only GSM symptoms). Exclusive administration of systemic estrogen is recommended for women who complain not only of genital symptoms but also of vasomotor disorders and problems related to osteoporosis [7]. Vaginal dryness and dyspareunia are the most common indications of low-dose local estrogen therapy, so the administration of low-dose vaginal estrogen is considered the gold standard for patients with vaginal atrophy and sexual dysfunction who have failed non-hormonal therapy. The therapeutic indication requires the application of the cream once per day for two weeks, followed by a maintenance dose of two to three applications per week. Ideally, women should be treated with the lowest dose and frequency with optimal symptom control that can be achieved [14]

Despite the availability of a variety of vaginal hormonal products, a recent systematic review showed that there is no clear evidence of differences in clinical efficacy when these therapies are compared with placebo [15]. Another problem related to vaginal estrogen is patient distrust of hormonal treatments and poor compliance with them. Daily application therapy often makes women abandon the use of this drug. Based on a 2013 survey of more than 3000 postmenopausal women regarding their experiences and perceptions of vaginally administered treatments, women reported high levels of dissatisfaction due to annoying application procedures and vaginal discharge following drug use [16, 17]. Compliance rates were reported to be only around between 52-74% [18]. In addition, topical administration of estrogen is also considered by some people not to have apparent long-term safety, especially considering the risk of estrogen exposure in the emergence of several cancers associated with high hormone levels. Recent research reports the side effects of increasing the risk of thrombosis, breast cancer and endometrial cancer from the use of topical estrogen [19].

An alternative hormonal therapy to vaginal estrogen is dehydroepiandrosterone (DHEA), the use of which intravaginally was recently approved by the Food and Drug Administration (FDA) for the treatment of GSM [20]. *Dehydroepiandrosterone* is an intermediate steroid hormone in the biosynthesis of androgens and estrogens and is effective in improving symptoms of vulvovaginal atrophy and restoring vaginal pH without causing harmful endometrial stimulation [12]. On the other hand, the only oral product approved to treat vaginal dryness and moderate to severe dyspareunia is Ospemifene, a selective estrogen receptor modulator with agonist or antagonist effects. A long-term efficacy and safety clinical study involving 180 women showed sustained improvement in symptoms and vaginal clinical examination, with no cases of endometrial hyperplasia or malignancy. However, until now, there is still no complete clarity about the possible side effects of Ospemifene because it has been proven to cause hot flashes and an increased risk of venous thromboembolism [21].

Laser and radiofrequency are alternative energy-based therapies developed for the new era of GSM treatment. This energy-based device is primarily intended for three groups of women with the following indications: (1) sufferers who have previously been treated with estrogen but whose symptoms have not improved, (2) sufferers with contraindications to the administration of estrogen, and (3) sufferers who refuse the administration of estrogen or the choice of therapy others but approve of energy-based device therapy [22, 23]. Lasers that are often used to treat vaginal atrophy are microablative fractional carbon dioxide (CO2) lasers and Erbium: YAG lasers [22]. The functional mechanisms of these two laser technologies and the tissue changes produced by them have led to their potential for treating vulvovaginal symptoms caused by hypoestrogenism [18].

Microablative CO2 laser for the vagina was introduced in 2014, with histological findings confirming its efficacy in transforming as well as rejuvenating vulvovaginal tissue in patients affected by GSM, while the effectiveness of the Erbium: YAG laser for vaginal atrophy was first reported in 2015 via a non-surgical thermal technique that produces hyperthermia vaginal collagen, thereby inducing remodeling and synthesis of new collagen fibers. Furthermore, this effect will result in increased firmness and elasticity of vaginal tissue, thereby improving symptoms of vaginal atrophy [9, 10].

Various studies on micro ablative fractional CO2 laser have been carried out on vaginal atrophy in menopausal women, both natural and iatrogenic menopause [18]. In vaginal tissue, micro ablative fractional CO2 laser has effects in the form of tissue remodeling, neovascularization, and re-production of

mucopolysaccharides by the extracellular matrix [19] Research by Athanasiou *et al*. suggested that micro ablative fractional CO2 laser also has beneficial effects on the vaginal microenvironment. This therapy is able to restore vaginal balance to what it was before menopause, namely when estrogen is still sufficient. Such conditions in the vagina can reduce the risk of infection and inflammation during menopause [24]. In addition, returning hydration to the vagina will normalize the vaginal pH to become more physiological, thus creating a protective layer that is a barrier to the entry of pathogenic microbes. Research by Paraiso *et al*. found that micro ablative fractional CO2 laser compared with standard intravaginal estrogen therapy had resulted in the effectiveness of both not being significantly different in improving symptoms, and patients were overall satisfied with laser administration without any significant side effects [18]

The use of micro ablative fractional CO2 laser in iatrogenic menopause has been widely studied in breast cancer survivor populations. This therapy is promising, considering that the use of hormonal therapy can increase the risk of cancer recurrence [25]. The theoretical basis for administering microablative fractional CO2 laser therapy in iatrogenic menopause is the same as the basis for using lasers in natural menopause, namely inducing tissue remodeling and vaginal architectural changes to become normal again [26].

Even though the research that has been conducted shows therapeutic efficacy and the increasing number of doctors' clinics that provide energy-based device services as a GSM therapy option, there are still few clinical trials available to validate its efficacy [7]. *The Food and Drug Administration* (FDA) in 2018 stated that there were still no recommendations for energy-based devices such as GSM therapy [27]. Therefore, more research is needed in the form of randomized controlled trials (RCT) and meta-analysis to evaluate and analyze the efficacy and safety of this technology so that it can be used as a recommendation [23].

Based on the description above, researchers are interested in conducting a systematic review and meta-analysis to analyze the effect of micro ablative fractional CO2 laser therapy compared with sham laser (sham laser) as a control on vaginal atrophy in menopausal women. This paper aims to conduct a systematic review, and this meta-analysis is to analyze the effect of micro ablative fractional CO2 laser therapy on vaginal atrophy in postmenopausal women.

2. Material and method

2.1. Research procedure

Data was collected online in the Pubmed-MEDLINE, Scopus, EBSCO, Cambridge Core, ProQuest, Springer Link, Cochrane Library, and ClinicalTrials.gov databases. Tracking will be carried out in May-July 2023. The population of this study is the result of clinical trial research on the effect of micro ablative fractional CO2 laser therapy on vaginal atrophy in postmenopausal women.

The sample of this study is a research report on the effect of microablative fractional CO2 laser therapy on vaginal atrophy in menopausal women in the study period who met the following criteria: (1) Research using microabative fractional CO2 laser with sham laser control; (2) In the form of a randomized controlled trial with randomization; (3) Research subjects experienced symptoms of vaginal atrophy both during menopause which occurred naturally and iatrogenically; (3) Research outcomes include VHI and VAS scores, and the incidence of side effects.

Exclusion criteria were as follows: (1) Research subjects with a history of using hormonal therapy since six months before the start of the study; (2) Research subjects with a history of using intravaginal estrogen, intravaginal lubricants or moisturizers since six months before the start of the study: (3) In the form of case reports, case series, letters, systematic reviews and literature reviews: (4) Articles written in languages other than Indonesian and English if there is no translated version.

The study sample size was all research reports regarding the effect of micro ablative fractional CO2 laser therapy on vaginal atrophy in postmenopausal women, which met the research criteria. The independent variables in this study were the administration of microablative fractional CO2 laser therapy and sham laser.

The independent variable in this study was vaginal atrophy in menopausal women with VHI and VAS score parameters. The following Medical Subject Headings (MeSH) terms were used to create two subgroups of citations (1) carbon dioxide laser; (2) CO2 lasers; (3) vaginal laser; (4) sham laser; (5) vaginal atrophy; (6) vulvovaginal atrophy; and (7) genitourinary syndrome of menopause. Subgroups were combined using the Boolean term 'OR' for subgroups (8) carbon dioxide laser, CO2 laser, vaginal laser, and (9) vaginal atrophy, vulvovaginal atrophy, genitourinary syndrome of menopause as well as 'AND' for combining with subgroup (8), (4) and (9) to obtain a subset of quotes that are relevant to the research question. The literature search was conducted based on the PRISMA 2009 flowchart. Three researchers conducted an independent literature search, and the reference lists of all primary articles and recent literature reviews were checked to identify any missing articles. Any disagreements in paper selection and data extraction were resolved by consensus.

Using a prepared data extraction form, three researchers extracted data independently. This form was created based on a modified data collection form from the Cochrane Library. The data recorded were characteristic and treatment data, including the effect of microablative fractional CO2 laser therapy and sham laser on VHI and VAS scores. Study quality assessment was carried out using the *Cochrane Risk of Bias Tool for Randomized Controlled Trials*.

2.2. Research Flow

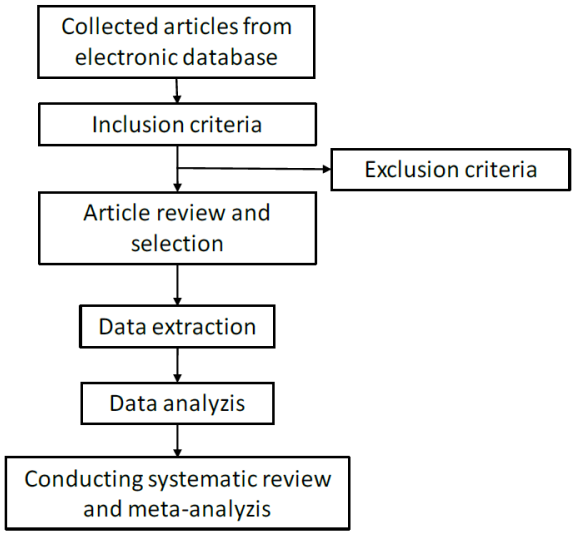

Fig 1: Research flow

2.3. Data Analysis

Before data analysis is carried out, the collected data will be checked for completeness and correctness of the data. The data will then be entered into the computer. The data extraction form records research characteristic data such as title, author, year, country, study population, length of treatment, and research design. Data in the form of microablative fractional CO2 laser therapy treatment or sham laser as well as VHI and VAS scores will be extracted from the research report and entered into the data extraction form.

Data analysis is divided into qualitative analysis using systematic reviews and quantitative analysis using meta-analysis. Qualitative analysis was carried out using data extraction that was considered necessary from articles that met the research criteria. Quantitative analysis (meta-analysis) was carried out by extracting data from articles that reported VHI and VAS values before and after laser therapy. Meta-analysis assessment uses the I2 statistical test. If the data is homogeneous (I2 value < 50%), then the meta-analysis will use a fixed effect model. The meta-analysis used mixed effect model analysis if the data is heterogeneous (I2 value ≥ 50%). Analysis was done using *Comprehensive Meta-Analysis: A Computer Program for Meta-analysis, Version 3.3.*

The publication bias was assessed using the funnel plot method and the Egger regression test. The risk of bias of the studies included in the analysis, both qualitative and quantitative analysis, was assessed using the Cochrane Risk of Bias Tool for Randomized Controlled Trials, including randomization methods, allocation concealment, blinding of research subjects, blinding of outcomes, incomplete outcome data, selection of reported outcomes, as well as other biases. The risk of bias assessment of each aspect is then converted according to the *Agency for Healthcare Research and Quality* (AHRQ) standards.

The quality of evidence for meta-analysis will be assessed using the *Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system*. The GRADE system assesses with a systematic approach that is transparent and structured, making it possible to develop recommendations that can be used as guidelines in clinical practice. GRADE can be assessed using *the software The GRADEpro Guideline Development Tool* (GDT).

3. Result

Data searches were carried out online at *Pubmed-MEDLINE, Scopus, EBSCO, ProQuest, Springer Link, Cochrane Library, ClinicalTrials.gov, and Google Scholar* with a time span until the analysis was carried out. From this search, 30 articles were found. We obtained eight relevant article titles after checking the titles and removing duplicates. The abstracts of these articles were then reviewed, resulting in 5 excluded articles, consisting of 1 title that needed a complete paper and four titles that needed complete data and met the inclusion criteria. Three articles in the form of complete papers were assessed for eligibility and used in a qualitative and quantitative study to assess the effect of microablative fractional CO2 laser on vaginal atrophy in postmenopausal women. The study literature search process can be seen in Figure 2.

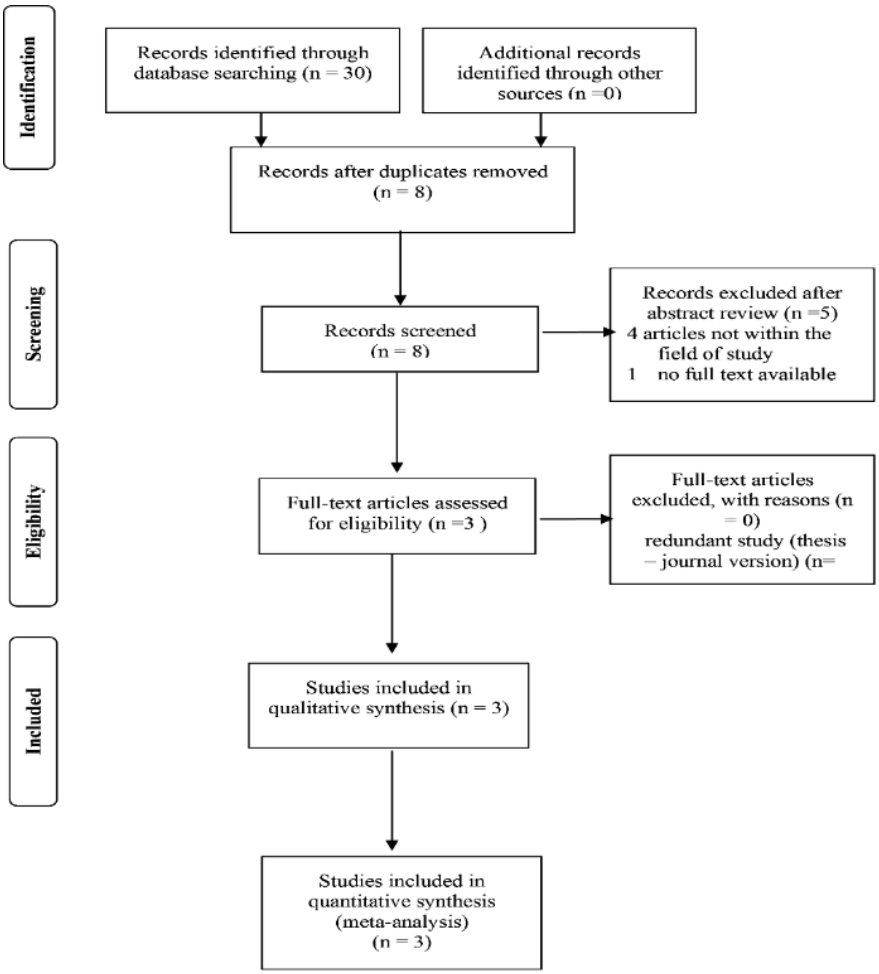

Fig 2: Flowchart of identification and selection of research literature in systematic reviews and meta-analyses

3.1. Study characteristics

Descriptive analysis of research characteristics consists of the country where it was carried out and the number of samples. The three studies all used postmenopausal female subjects who experienced vaginal atrophy with a total sample of 233 people. Three studies were conducted in Thailand, Australia, and Belgium. Ruanphoo's research was conducted from June 2016 to May 2017. Fiona's research was conducted from September 2016 to June 2019. Page's research was conducted from August 2019 to February 2020. The essential characteristics of the three studies are summarized in the table below.

Table 1: Research Characteristics

No	Researcher and year	Country	Number of samples	Treatment		Outcome	Frequency	Research design
				Treatment group	Control group			
1.	Ruanphoo, June 2016 to May 2017	Thailand	88	Laser fractional CO2 micro ablative	Sham laser	VHI, VAS, ICIQ-VS, side effect	Three laser sessions at a one-month interval	randomized double-blinded controlled trial
2.	Fiona, September 2016 hingga Juni 2019	Australia	85	Laser fractional CO2 micro ablative	Sham laser	VAS, VSQ, VHI	Three laser sessions at a one month interval	randomized double-blinded controlled trial
3.	Page, Agustus 2019 hingga Februari 2020	Belgia	60	Laser fractional CO2 micro ablative	Sham laser	MBS, VHI, VAS, FSFI, pH vagina, focal depth	Three laser sessions at a one month interval	randomized controlled trial

3.2. Results of Qualitative Data Analysis

1. Ruanphoo, *et al*

This research was conducted on 88 post-menopausal women who experienced vaginal atrophy, with 44 people in the treatment group and 44 people in the control group. The mean ± SD age of women was 60.78 ± 7.77 years. Age at menopause means ± SD was 49.21 ± 3.49 years. Thirty-four women (38.64%) were sexually active (10 [22.73%] in the treatment group and 24 [54.55%] in the control group).

Results measurements at baseline were shown; in the treatment group, the mean ± SD VHI and VAS were 14.18 ± 3.39 and 2.27 ± 0.42, respectively. In the control group, the mean ± SD VHI and VAS were 14.66 ± 2.91 and 2.02 ± 0.40, respectively. Data were then compared between baseline and follow-up at week 12. In the treatment group, there was a significant improvement for all parameters. The VHI score improved significantly from 14.18 ± 3.39 at baseline to 17.45 ± 2.61 at week 12, P < 0.001. VAS scores decreased from 2.27 ± 0.42 to 1.83 ± 0.51, P < 0.001. ICIQ-VS score, for vaginal dryness significantly decreased from 5.00 (2.00-6.00) to 3.24 (0-4.00), P = 0.02. In the control group, there was no statistical difference in VHI scores after 12 weeks (P=0.06). VAS scores increased from 2.02 ± 0.40 at baseline to 2.06 ± 0.49 at week 12 of observation (P=0.59). In ICIQ-VS, vaginal dryness decreased at week 12 (P=0.07). Reported side effects include vaginal bleeding, vaginal discharge, vaginitis, and pain after the procedure. Side effects did not differ statistically significantly between the two groups.

2. Fiona, *et al*

This research was conducted on 85 post-menopausal women who experienced vaginal atrophy. There were no significant differences between the treatment and control groups from baseline to 12 months in changes. VAS scores for overall symptoms were -17.2 and -26.6, respectively, 95% CI, and for the most severe symptoms were -24.5 and -20.4, respectively, 95% CI. There was no significant difference between the treatment and control groups from baseline to 12 months for total VSQ scores (3.1 and -1.6 respectively; CI 95%). There was no statistically significant difference in VHI between the treatment and control groups, respectively 0.9 and 1.3 IK 95%. Overall, 16 people in the treatment group and 17 people in the control group reported vaginal pain that resolved on its own or discomfort (44% vs 68%), spotting, urinary tract infections and vaginal discharge.

3. Page, *et al*

This research was conducted on 60 post-menopausal women who experienced vaginal atrophy. The MBS score at the end of the observation decreased from 2.86 ± 0.35 to 2.17 ± 0.93 (CI 95%) in the microablative fractional CO2 laser group compared to the sham laser group which at the beginning of the observation was 2.90 ± 0.31 to 2.52 ± 0.78 (CI 95%) at the end of observation. No serious side effects were reported in that study after up to 18 months of observation.

The results of research by Ruanphoo *et al*. in 2020 showed that the VHI score in the micro ablative fractional CO2 laser group was significantly higher than the VHI score in the sham laser group (p=0.036). Page *et al*.'s 2022 research discovered that the VHI score of the microablative fractional CO2 laser group was also higher than the sham laser group (p=0.143). In research by Fiona, *et al* in 2021, it was found that the VHI score of the microablative fractional CO2 laser group was lower than the sham laser group, but the difference was not significant (p-0.895). The results of research by Ruanphoo *et al*. in 2020 found that the reduction in VAS scores in the micro ablative fractional CO2 laser group was significantly more significant than in the sham laser (p<0.001). In a study by Fiona *et al*. in 2021, the reduction in VAS scores in the micro ablative fractional CO2 laser group was also more significant than in the sham laser group (p=0.079). In a study by Page *et al*. in 2022, it was found that the decrease in VAS scores in the micro ablative fractional CO2 laser group was actually smaller than in the sham laser group, but this difference was not significant (p-0.734).

The incidence of side effects based on the research results of Ruanphoo *et al*. in 2020 was more in the micro ablative fractional CO2 laser group, but this difference was not significant (p=0.878). In contrast between Fiona *et al*. in 2021 and Page *et al*. in 2022, the incidence of side effects in the micro ablative fractional CO2 laser group was less than in the sham laser group, but this difference was not significant (Fiona 2022 p=0.757; Page 2022 p=0.453).

3.3. Meta-Analysis Results of the Effect of Microablative Fractional CO2 Laser on VHI

The difference in mean VHI scores before and after administering fractional CO2 laser therapy and sham laser is shown in Table 2.

Table 2: VHI scores in the fractional CO2 laser group and sham laser group

Study name	Group VHI Score			
	Laser CO2		SHAM Laser	
	Rarity ±SB	n	Rarity ±SB	n
Ruanphoo P, 2020	3.27±3.35	44	1.42±4.71	44
Fiona G, 2021	14.1±3.47	42	14.2±3.43	41
Page AS, 2022	2.9±4.71	29	1.2±4.01	29
Note: the numbers in the table are the difference between the mean and standard deviation (SB) before and after treatment				

Table 2 shows positive difference in mean VHI scores before and after fractional CO2 laser therapy. This shows an increase in VHI scores in vaginal atrophy in menopausal women after therapy with fractional CO2 laser. The control group also showed that the difference in mean VHI scores after administering the sham laser was all positive. This shows that after administering sham laser therapy in the control group, there was also an increase in the VHI score. Ruanphoo *et al*.'s research showed that the VHI score after administering fractional CO2 laser was 3.27 ± 3.35, showing a higher increase than the sham laser control, namely 1.42 ± 4.71. In Fiona *et al*'s research, the VHI score after administering fractional CO2 laser was 14.1 ± 3.47, showing almost the same increase as the sham laser control, namely 14.2 ± 3.43. Page *et al*'s research showed that the VHI score after administering fractional CO2 laser was 2.9 ± 4.71, higher than the sham laser control, 1.2 ± 4.01.

The results of the meta-analysis comparing changes in VHI scores before and after microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women are shown in Figure 3.

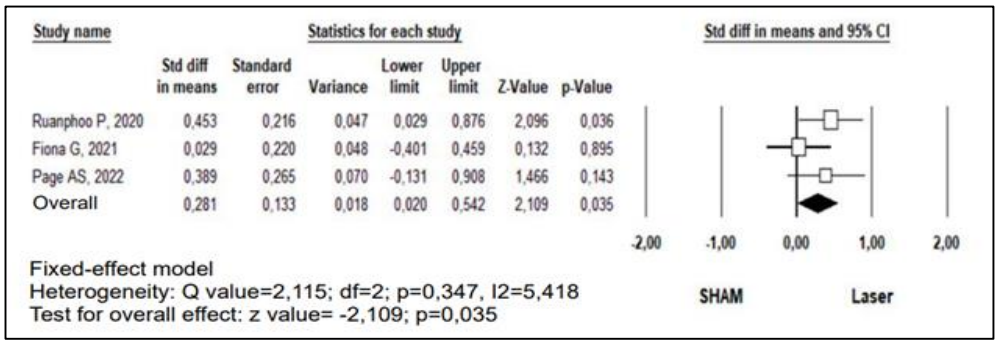

Fig 3: Results of meta-analysis comparing changes in VHI scores before and after microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women

In Figure 3, the results of a meta-analysis of the effect of microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women are displayed. The heterogeneity test results show the Q value = 2.115; df=2; p=0.347, I2=5.418. This shows that the data is homogeneous, in line with the results of the statistical Q test and heterogeneity, the I2 test results obtained < 50%. This shows that the data is homogeneous, so the analysis is carried out using a fixed effect model. The meta-analysis results show that the statistical Q value is z value = -2.109; p=0.035. This shows that overall microablative fractional CO2 laser administration can significantly improve the VHI score in vaginal atrophy in postmenopausal women.

The overall meta-analysis results show that the overall standardized mean difference in VHI scores between subjects who received microablative fractional CO2 laser compared to those who received sham laser was 0.281 ± 0.133 (95% CI, 0.020 to 0.542). These results show that the overall increase in the VHI score of the group that received the microablative fractional CO2 laser was significantly higher than that of the overall sham laser (p=0.035).

3.4. Effect of Microablative Fractional CO2 Laser on VAS

The difference in mean VAS scores before and after administering fractional CO2 laser therapy and sham laser is shown in Table 3.

Table 3: VAS scores in the micro ablative fractional CO2 laser group and the sham laser group

Study name	Group VAS Score			
	Laser CO2		SHAM Laser	
	Rarity ±SD	n	Rarity ±SD	n
Ruanphoo P, 2020	-0.44±0.51	44	0.04±0.51	44
Fiona G, 2021	-1.68±2.73	42	-0.7±2.27	41
Page AS, 2022	-0.978±1.45	29	-1.106±1.42	29

Table 3 shows a negative difference in mean VAS scores before and after fractional CO2 laser therapy. This shows a decrease in VAS scores in vaginal atrophy in menopausal women after therapy with fractional CO2 laser. The control group also showed differences in the mean VAS scores after administering the sham laser, including positive and negative values. This shows that after giving sham laser therapy, some in the control group experienced a decrease in VAS scores, but some did not. Ruanphoo *et al*.'s research showed that the VAS score after administering fractional CO2 laser, namely -0.44 ± 0.51, decreased compared to the sham laser control, namely 0.04 ± 0.51. In Fiona *et al*'s research, the VAS score after administering fractional CO2 laser, namely -1.68 ± 2.73, showed a more significant reduction than the sham laser control, namely -0.7 ± 2.27. In-Page *et al*.'s research, the VAS score after administering a fractional CO2 laser was -0.978 ± 1.45, showing a lower reduction than the sham laser control, namely -1.106 ± 1.42.

The results of the meta-analysis comparing changes in VAS scores before and after microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women are shown in Figure 4.

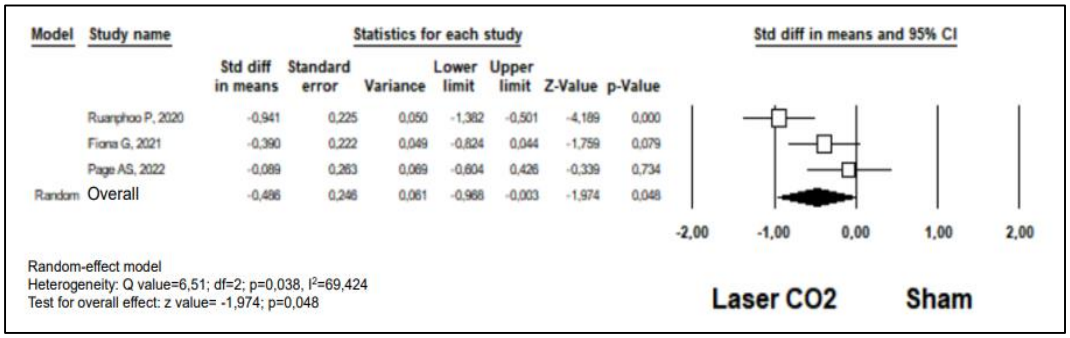

Fig 4: Results of meta-analysis comparing changes in VAS scores before and after micro ablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women

Figure 4 shows the results of a meta-analysis of the effect of micro ablative fractional CO2 laser therapy with sham laser as a therapy for vaginal atrophy in menopausal women. Heterogeneity test results show Q value = 6.51; df=2; p=0.038, I2=69.424. This shows that the data is not homogeneous, in line with the results of the statistical Q test and heterogeneity, the I2 test results obtained > 50%. This also shows that the data is not homogeneous, so the analysis was carried out using a random effect model. The meta-analysis results show that the statistical Q value is z value = -1.974; p=0.048. This shows that overall micro ablative fractional CO2 laser administration can significantly reduce VAS scores in vaginal atrophy in menopausal women.

The overall meta-analysis results show that the overall standardized mean difference in VAS scores between subjects who received microablative fractional CO2 laser compared to those who received sham laser was -0.510 ± 0.134 (95% CI, -0.775 to -0.245). These results show that the overall VAS score of the group that received the microablative fractional CO2 laser was significantly reduced compared to the overall sham laser (p= 0.000).

3.5. Meta-Analysis Results of the Effect of Microablative Fractional CO2 Laser on Side Effects

The difference in the incidence of side effects before and after administering fractional CO2 laser therapy and sham laser is shown in Table 4.

Table 4: The incidence of side effects in the micro ablative fractional CO2 laser and sham laser groups

Study Name	Laser CO2		Sham laser		Risk Ratio
	Side effect occurrence	n	Side effect occurrence	n	
Ruanphoo P, 2020	7 (17.1)	41	6 (15.8)	38	1.1
Fiona G, 2021	16 (37.2)	43	17 (40.5)	42	0.9
Page AS, 2022	3 (10.3)	29	5 (17.2)	29	0.6
Note: The incidence of side effects is expressed as n (%)					

Table 4 shows positive difference in mean VHI scores before and after fractional CO2 laser therapy. This shows an increase in VHI scores in vaginal atrophy in menopausal women after therapy with fractional CO2 laser. The control group also showed that the difference in mean VHI scores after administering the sham laser was all positive. This shows that after administering sham laser therapy in the control group, there was also an increase in the VHI score. Ruanphoo *et al*.'s research showed that the VHI score after administering fractional CO2 laser was 3.27 ± 3.35, showing a higher increase than the sham laser control, namely 1.42 ± 4.71. In Fiona *et al*'s research, the VHI score after administering fractional CO2 laser was 14.1 ± 3.47, showing almost the same increase as the sham laser control, namely 14.2 ± 3.43. Page *et al*'s research showed that the VHI score after administering fractional CO2 laser was 2.9 ± 4.71, higher than the sham laser control, 1.2 ± 4.01.

The results of a meta-analysis comparing the incidence of side effects before and after microablative fractional CO2 laser therapy with sham laser as a therapy for vaginal atrophy in menopausal women are shown in Figure 5.

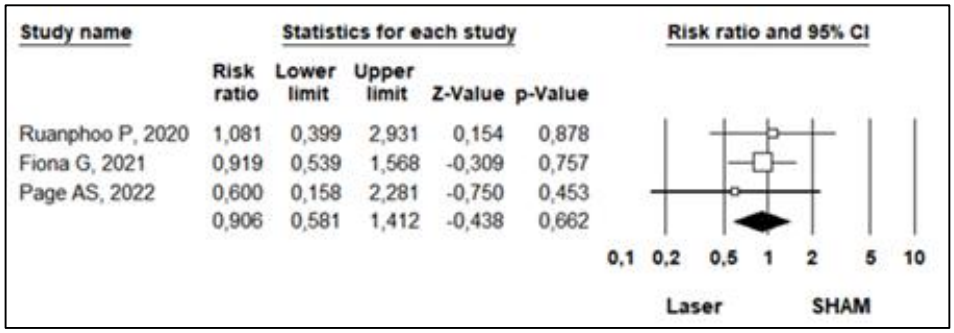

Fig 5: Results of meta-analysis comparing the incidence of side effects before and after microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women. Note: Fixed-effect model Heterogeneity: Q value = 0.489; df = 2; p = 0.783. I2=0.000 Test for overall effect: z value= -0.438; p=0.662

In Figure 5, the results of a meta-analysis comparing the incidence of side effects before and after administration of microablative fractional CO2 laser therapy with sham laser as therapy for vaginal atrophy in menopausal women are displayed. Heterogeneity test results show Q value = 0.489; df=2; p=0.783, I2=0.000. This shows that the data is homogeneous, in line with the results of the statistical Q test and heterogeneity; the I2 test results obtained insignificant results, indicating that the data is homogeneous, so the analysis was carried out using a fixed effect model. The meta-analysis results show that the statistical Q value is z value = -0.438; p=0.662.

The overall meta-analysis results show that the overall risk ratio for side effects between subjects who received microablative fractional CO2 laser compared to those who received sham laser was 0.906 (95% CI, 0.581 to 1.412). This shows that overall the difference in the incidence of side effects was insignificant in the microablative fractional CO2 laser group compared to the sham laser group (p=0.662).

3.6. Risk of Bias Assessment

The risk of bias of the studies included in the analysis, both qualitative and quantitative analysis, was assessed using the Cochrane Risk of Bias Tool for Randomized Controlled Trials, including randomization methods, allocation concealment, blinding of research subjects, blinding of outcomes, incomplete outcome data, selection of reported outcomes, as well as other biases. Each aspect's risk of bias assessment was then converted according to Agency for Healthcare Research and Quality (AHRQ) standards. Assessment of the risk of bias in studies of the effect of micro ablative fractional CO2 laser therapy with sham laser on vaginal atrophy in postmenopausal women used in the meta-analysis is shown in Table 5.

Table 5: Assessment of research risk of bias

Author	Random sequence generation	Allocation concealment	Blinding (participants and personnel)	Blinding (outcome assessment)	Selective reporting	Other sources of bias	Incomplete outcome data	Other bias	Overall
Ruanphoo P, 2020	+	+	+	+	+	+	+	+	+
Fiona G, 2021	+	+	+	+	+	+	+	+	+
Page AS, 2022	+	+	+	+	+	+	+	+	+

Note: + = low risk of bias

In all assessment categories, the three studies, Ruanphoo, Fiona and Page, had a low risk of bias.

3.7. Quality of Evidence Evaluation

The quality of evidence assessment for this meta-analysis was carried out using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system. The GRADE system assesses with a systematic approach that is transparent and structured, making it possible to develop recommendations that can be used as guidelines in clinical practice. GRADE can be assessed using The GRADEpro Guideline Development Tool (GDT) software.

The GRADE assessment begins by entering a specific problem and outcome formulation. After all assessment results are collected and concluded, GRADE can provide an assessment of the quality of evidence for systematic reviews and meta-analyses by assessing several aspects, including the number and design of included studies, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect size, confidence that the effects obtained can be recommended and the importance of the outcome assessment used.

The resulting recommendations have four levels of evidence, namely very low, low, medium and high. Very low means the actual effect may be very different from the predicted effect, low means the actual effect may be very different from the predicted effect, medium means the author believes that the actual effect may be close to the predicted effect, and high means the author is very confident that the actual effect may be close to the predicted effect. which is estimated.

Quality of evidence meta-analysis of the effect of micro ablative fractional CO2 laser therapy and sham laser as therapy for vaginal atrophy in menopausal women is shown in the table

Quality of evidence assessment for VHI, VAS scores, and side effects in all categories were assessed as not serious. Thus, the results of the quality of evidence assessment based on GRADE are high.

3.8. Publication Bias Risk Assessment

The quality of evidence assessment for this meta-analysis was carried out using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system. The GRADE system assesses with a systematic approach that is transparent and structured, making it possible

to develop recommendations that can be used as guidelines in clinical practice. GRADE can be assessed using The GRADEpro Guideline Development Tool (GDT) software.
The GRADE assessment begins by entering a specific problem and outcome formulation. After all assessment results are collected and concluded, GRADE can provide an assessment of the quality of evidence for systematic reviews and meta-analyses by assessing several aspects, including the number and design of included studies, risk of bias, inconsistency, indirectness, imprecision, number of patients, effect size, confidence that the effects obtained can be recommended and the importance of the outcome assessment used.
The resulting recommendations have four levels of evidence, namely very low, low, medium and high. Very low means the actual effect may be very different from the estimated effect, low means the actual effect may be different from the estimated effect, medium means the author believes that the actual effect is probably close to the predicted effect, and high means the author is very confident that the actual effect is probably close to the predicted effect. Quality of evidence meta-analysis of the effect of micro ablative fractional CO2 laser therapy and sham laser as therapy for vaginal atrophy in menopausal women is shown in table 6.

Table 6: Analysis of quality of evidence meta-analysis of the effect of micro ablative fractional CO2 laser therapy and sham laser as therapy for vaginal atrophy in menopausal women

Study	Number of subjects	Quality assessment					Summary of findings	
		Risk of bias	Inconsistency	Indirect-ness	Imprecision	Publication bias	Effect size (IK 95%)	Quality of evidence
Score VHI	3 (Laser CO2 n=115, SHAM n=114)	Not serious	None	Direct	No impression	None	Mean different= 0.281± 0.134 (95% IK= 0.020 s/d 0542)	++++ (high)
Score VAS	3 (Laser CO2 n=115, SHAM n=114)	Not serious	None	Direct	No impression	None	Mean different= -0.510±0.134 (95% IK= -0.775 s/d 0.245)	++++ (high)
Side effects	3 (Laser CO2 n=115, SHAM n=109)	Not serious	None	Direct	No impression	None	Risk Ratio 0.906 (95% IK=0.581 s/d 1.412)	++++ (high)

A funnel diagram plot of the risk of bias assessment is shown in Figure 7

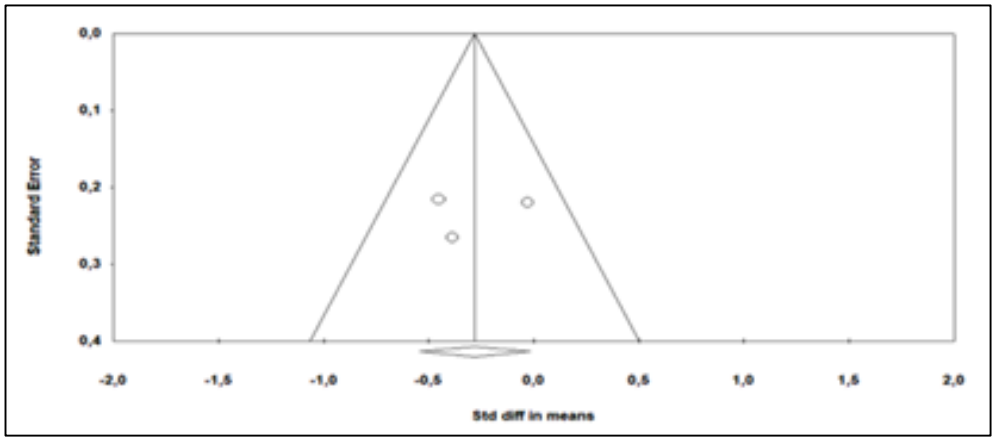

Fig 7: Funnel plot of risk of bias for publication meta-analysis of the effect of micro ablative fractional CO2 laser therapy and sham laser as therapy for vaginal atrophy in menopausal women

In Figure 7, the funnel plot diagram shows a symmetrical picture. Analysis using the Egger regression test was not significant (t value= 0.259; p=0.839). Based on the symmetrical funnel plot diagram and Egger's regression test, which was insignificant, it showed no risk of publication bias.

4. Discussion

This study is a systematic review and meta-analysis study regarding the effect of microablative fractional CO2 laser therapy on VHI and VAS score parameters.
Regarding the VHI score, of the three articles used, two of them, namely Ruanphoo *et al*.'s research in 2020 and Page *et al*. in 2022, there was an increase in VHI. This is in accordance with the hypothesis that the VHI score in the group that received microablative fractional CO2 laser therapy was higher than the VHI score in the group that received sham laser therapy for vaginal atrophy in menopausal women. In one research article, namely by Fiona *et al*. in 2021, the VHI score in the group that received microablative fractional CO2 laser therapy was lower than the VHI score in the group that received sham laser therapy for vaginal atrophy in menopausal women, but this difference was not significant.
In the research of Ruanphoo *et al*., 2020 and Page, *et al*., 2021, the VHI value increased compared to the sham laser group. This is in accordance with previous research conducted by Salvatore *et al*. in 2014, reporting the results of a 12-week evaluation after CO2 laser therapy for vulvovaginal atrophy in 50 post-menopausal women, showing a significant increase in VHI scores compared to baseline [36]. In Sokol *et al*. in 2015 study using 30 female subjects who experienced GSM after receiving three laser sessions at intervals of 6 weeks, there was an improvement in the VHI score [37].
In a study by Fiona *et al*. in 2021, the increase in VHI scores in the group that received micro ablative fractional CO2 laser therapy was lower than the VHI score in the group that received sham laser therapy, but this difference was not

significant. The results of this study are similar to the RCT research conducted by Cruff *et al* in 2016, which was conducted with subjects of natural and iatrogenic vaginal atrophy in menopausal women. It was found that microablative fractional CO2 laser was given three times with six week intervals in both groups, both microablative fractional CO2 laser groups and the sham laser group both experienced an increase in VHI scores but the difference was not significant. This is possibly due to the placebo effect [38]. In research conducted on experimental animals, menopausal vaginal biopsies that underwent sham laser therapy were previously found to have an effect that could be measured through histopathological examination [36].

Regarding the VAS score, of the three articles used, two of them, namely the research by Ruanphoo *et al*. in 2020 and Fiona *et al*. in 2021. The decrease in VAS score in the microablative fractional CO2 group was more significant than in the sham laser group. This is in accordance with the hypothesis that the VAS score in the group that received microablative fractional CO2 therapy was lower than the group that received sham laser therapy for vaginal atrophy in menopausal women. However, in another article used, namely research by Page *et al*. in 2022, the decrease in VAS scores in the micro ablative fractional CO2 group was smaller than in the sham laser group, although this difference was not significant. In a previous observational study conducted by Sokol *et al*. in 2015, there was an improvement in VAS scores for all symptoms assessed, including pain, vaginal burning, vaginal itching, vaginal dryness, dyspareunia, and dysuria [39].

The results of the meta-analysis show that overall microablative fractional CO2 laser administration can significantly improve the VHI score in vaginal atrophy in postmenopausal women. The results of the meta-analysis showed that the overall standardized mean difference in VHI scores between subjects who received microablative fractional CO2 laser compared to those who received sham laser was 0.281 ± 0.133 (95% CI, 0.020 to 0.542). These results show that the increase in the VHI score of the group receiving the microablative fractional CO2 laser was significantly higher than the overall sham laser. This is in accordance with a meta-analysis research conducted by Meichen, *et al* in 2021 from 12 articles studied which stated that CO2 laser administration experienced a significant increase in VHI at 1, 3, 6, and 12 months when compared with baseline [34]. Meta research Another analysis conducted by Filippini *et al*. in 2022 from 25 articles with GSM female subjects showed an increase in VHI scores after being given CO2 laser therapy [35].

The results of the meta-analysis show that overall administration of microablative fractional CO2 laser can significantly reduce VAS scores in vaginal atrophy in menopausal women. The meta-analysis results showed that the overall standardized mean difference in VAS scores between subjects who received microablative fractional CO2 laser compared to those who received sham laser was -0.510 ± 0.134 (95% CI, -0.775 to -0.245). These results show a significant reduction in the VAS score of the group that received the microablative fractional CO2 laser compared to the overall sham laser. A meta-analysis study comparing microablative fractional CO2 laser therapy with sham laser showed no significant difference in VAS scores between the groups. In a meta-analysis study conducted by Jang *et al*. in 2022, which compared micro ablative fractional CO2 laser therapy with topical estrogen, there was no significant difference in VAS scores between the groups [36].

The results of the meta-analysis showed that the overall risk ratio for side effects between subjects who received microablative fractional CO2 laser compared to those who received sham laser was 0.906 (95% CI, 0.581 to 1.412). This shows that overall the difference in the incidence of side effects is insignificant in the microablative fractional CO2 laser group compared to the sham laser group. These results are in accordance with previous meta-analysis research conducted by Jang *et al*. in 2022, which stated that micro ablative fractional CO2 laser administration did not cause serious side effects [36].

Over the last decade, the use of CO2 lasers has been widely used in the medical field. This laser consists of infrared rays that produce heat and evaporate water as a target. The mechanism of action of the microablative fractional CO2 laser begins with thermo-ablative damage, followed by proliferation. The laser will stimulate the synthesis of collagen and matrix component substances in the irradiated area so as to achieve regeneration, which will increase the elasticity and hydration of the vaginal walls and eliminate discomfort in menopausal women.[2]

Symptoms of vaginal atrophy have a negative impact on quality of life and affect sexual life with one's partner. Hormonal therapy often causes side effects, especially in women with a history of breast cancer, so technologies such as lasers are starting to be developed [37]. However, according to the statement issued by the FDA in 2018 regarding the lack of evidence regarding the benefits and risks of harm from energy-based devices, doctors must remain careful in using them as GSM therapy [30]. This is because the CO2 laser is a minimally invasive procedure hence the effects can occur. Side effects such as vaginal lacerations, scar tissue or perforation still need to be watched out for the treatment [30, 36].

5. Research Limitations

Researchers realize that in this research, there are still limitations, namely

1. The number of articles and research subjects still needs to be increased.
2. The duration of the study is short so clinically it is not known the effect of administering micro ablative fractional CO2 laser on vaginal atrophy in long-term menopausal women

6. Conclusion

Based on systematic review and meta-analysis data, it can be concluded that

1. The VHI score was higher after therapy in the group that received microablative fractional CO2 laser compared to the group that received sham laser for vaginal atrophy in menopausal women.
2. VAS scores were lower after administration in the group that received microablative fractional CO2 laser compared to the group that received sham laser for vaginal atrophy in menopausal women.
3. The incidence of side effects in the CO2 laser group was no different from the sham laser group.

7. Recommendation

1. An RCT study was conducted comparing micro ablative fractional CO2 laser compared to other energy-based

devices, such as erbium laser and radiofrequency on vaginal atrophy in postmenopausal women so that it can be used as a basis for further systematic review research and meta-analysis.
2. It is recommended that the duration of therapy in future studies be longer so that we can assess the long-term effects of microablative fractional CO2 laser on vaginal atrophy in menopausal women.

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