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Vaginal natural oxygenation device (VNOD) for concomitant administration of hyaluronic acid and topical hyperbaric oxygen to treat vulvo-vaginal atrophy: a pilot study

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Abstract. – OBJECTIVE: This is a pilot study to evaluate the effectiveness of concomitant administration of hyaluronic acid and topical hyperbaric oxygen therapy (THOT) by a specifically designed medical device (vaginal natural oxygenation device, VNOD) in improving the symptomatology of postmenopausal patients with vulvo-vaginal atrophy (VVA).

PATIENTS AND METHODS: Women with diagnosis of severe VVA from September 2017 to May 2018 were included. Five biweekly administration of THOT and concomitant of hyaluronic acid were performed with a specifically designed medical device. In each occasion, the intensity of patient's symptoms (well-being such as absence of dyspareunia, vaginal dryness, vulvar and/or vaginal itching; vaginal burning; presence of fluid) was determined with a graduated scale from 1 to 6 and the vaginal elasticity and the vaginal wall epithelium appearance were also determined with a graduated scale from 1 to 5. The change in all parameters from baseline to end of therapy was evaluated.

RESULTS: Twenty-five patients were considered for the final analysis. A significant improvement in well-being (0.3 *vs.* 5.1, $p < 0.001$), vaginal burning (0.2 *vs.* 5.1, $p < 0.001$), presence of fluid (0.6 *vs.* 4.9, $p < 0.001$), vaginal epithelium appearance (1.8 *vs.* 4.7, $p < 0.001$), and vaginal elasticity (1.1 *vs.* 3.8, $p < 0.001$) was observed between the first and the last therapy session. All the patients reported a recovery of their sexuality at the end of the five treatment sessions.

CONCLUSIONS: In this pilot study, the use of VNOD seems to be a valid treatment of VVA, resulting in a completely natural type of therapy well accepted by patients with immediate therapeutic effects and without side effects; these findings must be confirmed in a well-designed randomized controlled trial.

Key Words:
Vulvo-vaginal atrophy, Topical hyperbaric oxygen, Hyaluronic acid, Dyspareunia, Genitourinary syndrome of menopause.

Introduction

Menopause is commonly associated with somatic symptoms, including hot flashes, night sweats and fatigue, but women are less frequently aware of vulvovaginal symptoms, including vulvo-vaginal dryness and atrophy, recurrent urinary tract infections, and dyspareunia. Postmenopausal vulvar and, vaginal atrophy (VVA) is characterized by the thinning, drying, and loss of elasticity of the vaginal epithelium associated with the reduction in serum estrogen levels[1]. VVA can be diagnosed because of symptoms reported by the patient and by clinical examination[2,3]. These symptoms are presents up to 50% of postmenopausal women, including vaginal dryness, irritation, itching, dysuria, and pain or bleeding with sexual activity[4,5]. Based on the increase in life expectancy, most of the women can live almost 40% of life after menopause, and because the VVA is progressive without treatment, it can negatively significantly

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affect the quality of life[6]. The loss of estrogenic production by the ovary is associated with the onset of vaginal atrophy and results in decreased vaginal lactobacilli, increased pH, alteration of the epithelium morphology, reduction of blood flow and vaginal fluid secretion. The loss of vaginal wrinkled folds and the thinning of the epithelium occurs about 2-3 years after menopause with a variable onset of these physical signs. The loss of roughness is the consequence of an alteration of the collagen supporting the vaginal epithelium. The first-line therapies recommended by the North American Menopause Society include vaginal moisturizers, continuous sexual activity and lubricants[7]. When symptoms persist after taking first-line therapies, vaginal local estrogenic therapies (LET) are considered effective and well tolerated for the treatment of moderate to severe symptomatic VVA due to minimal systemic absorption, and are currently recommended compared to systemic estrogenic therapy when VVA is the only pathology. However, estrogenic therapy is used and continued by only about 2% of women. Recently, various solutions have been proposed such as hyaluronic acid in cream[8,9], vaginal laser[10,11], or injection of autologous adipose tissue[12], with varying results in terms of clinical effectiveness. To develop new therapeutic approaches to the treatment of VVA, molecular oxygen could have promising characteristics to counteract the main modifications of tissue atrophy and hypoxia-related to this condition. Oxygen therapy increases the availability of oxygen to the tissues, promotes the increase of the reparative processes of the tissues, increases the synthesis of collagen and induces a neo-angiogenetic stimulation through the release of the Vascular Endothelial Growth Factor (VEGF). The hyperbaric oxygen can be administered topically, directly on the skin or mucous membranes affected, using particular devices (topical hyperbaric oxygen therapy, THOT). The THOT could be used in combination with hyaluronic acid, in patients who have contraindications for hormone treatment or in women who wish to use non-hormonal and non-invasive methods.

The aim of this pilot study was to evaluate the effectiveness of concomitant administration of hyaluronic acid and THOT by a specifically designed medical device (Vaginal Natural Oxygenation Device, VNOD) in improving the symptomatology of postmenopausal patients with VVA.

Patients and Methods

Patients

A series of women with diagnosis of VVA based on symptoms reported and clinical examination from September 2017 to May 2018 were considered. The severity of vaginal atrophy was assessed using a Visual Analogue Scale (VAS), based on a four-point scale. Patients indicated the intensity of the perceived symptom using a score variable from 0 (no symptom) to 3 (presence of the symptom with maximum intensity). The present study included only patients reporting a score of 3 (maximum intensity).

Women who had not performed a cervical cytology in the last year, those with presence of vaginal infections and patients who had concomitant clinically important medical disease (endometrial hyperplasia or cancer; undiagnosed vaginal bleeding; liver or kidney disorder; thromboembolic disorders; cerebrovascular accident, stroke, or transient ischemic attack; myocardial infarction or ischemic heart disease; malignancy; endocrine disease or any clinically important abnormalities on screening physical examination, assessments, mammogram, electrocardiogram (ECG), or laboratory tests), were excluded. Women who used oral products containing estrogens, progestins, androgens, or selective estrogen receptor modulators (SERMs) within 8 weeks, transdermal hormone products within 4 weeks, vaginal hormone products (rings, creams, gels) within 4 weeks, intrauterine progestins within 8 weeks, progestin implants/injectables or estrogen pellets/injectables within 6 months, an intrauterine device within 12 weeks before screening, vaginal lubricants and moisturizers within 8 weeks, were excluded.

For the treatment, the self-cooling X2 (Exea - MDM Industrial srl, Padulle, Bologna, Italy) device was used composed of a compressor unit and a base unit (generator) able to deliver up to 6 l/minute of 95% pure oxygen. The base unit was equipped with an on-board computer and a graphic-touchscreen interface that allowed the flow modulation. The full treatment cycle includes five biweekly sessions, during which the single use dispenser was inserted through the vagina for 15 minutes, and the hyperbaric oxygen and the hyaluronic acid were administered at first alternately and subsequently in a contemporary way. The treatment began with the delivery of 95% pure oxygen through a cannula specifically designed for vaginal therapy. The oxygen was

delivered at a flow of 2 lt/ minute for 15 minutes; in the last 5 minutes a solution of low molecular weight sodium hyaluronate, at a concentration of 0.2%, was administered. At visit 0, an accurate collection of patient history and a complete clinical examination was performed to evaluate eligibility criteria. The intensity of the three following symptoms (1. well-being such as absence of dyspareunia, vaginal dryness, vulvar and/or vaginal itching, 2. vaginal burning 3. vaginal lubrication and presence of fluid) were collected from patients with a VAS based on a six-point scale: score 1 = maximum intensity, score 2 = strong intensity, score 3 = average intensity, score 4 = mild intensity, score 5 = weak intensity and score 6 = absence of symptom (Table I). Before the treatment, the vaginal elasticity and the vaginal wall epithelium appearance were determined by the clinician with a numerical score as reported in Table II. The first 15 minutes session was performed and the occurrence of any discomfort or adverse effect was recorded. The next four administrations (visit 1, 2, 3 and 4) were performed after 14 days. On every occasion, the intensity of patient's symptoms, the vaginal elasticity, and the vaginal wall epithelium appearance were determined and recorded with the method described above, as well as the occurrence of any adverse event. Follow-up visit was performed after 30 days from the last administration and even on those occasions the vaginal elasticity, the vaginal wall epithelium appearance and the intensity of patient's symptoms were determined and recorded. Patients were identified and recruited from investigators clinics and referring physician, with privacy protection and avoiding undue influence. Each included patient provided an informed consent that allowed treatments, that certified the comprehension of the information provided, with voluntary agreement of the subject, free from coercion. The treatments were administered at Ospedale Civile Urbino – SSD Oncologia Ginecologica; the Local Ethical Committee approval was obtained.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 22.0 (IBM Corporation, Armonk, NY, USA). The statistical significance of the trend of variation in values between treatment sessions was analyzed using the one-way variance analysis according to the Kruskal-Wallis method. The significance of couples' comparisons between treatment sessions was analyzed using the Wilcoxon test for non-parametric data. A $p < 0.05$ was considered statistically significant.

Results

The mean age at diagnosis of the 25 included cases was 56.6 years ± 6.8 SD (range 44 - 66). Ten patients were excluded for the presence of at least one of the factors reported above. Five included patients underwent previous hysterectomy (in one case for ovarian cancer and in four cases for benign diseases). Three patients reported previous surgical and medical treatments for breast cancer. No patient discontinued therapy, performing less than five sessions. One patient reported a slight bleeding after the first treatment following sexual intercourse, probably due to neo-vascularization of the tissue. This side effect was not reported after subsequent treatments. The results shown a significative improvement of all the mean scores analyzed: well-being such as absence of dyspareunia, vaginal dryness, vulvar and/or vaginal itching, vaginal burning, vaginal lubrication and presence of fluid (Figure 1). The intensity of the three symptoms reported according to the 6-point VAS scale (Table I) showed an average increase of the well-being index from 0.3 to 5.1, an average increase of the burning index 0.2 to 5.1 and of the fluidity index from 0.6 to 4.9. On a 5-point scale (Table II), the average epithelial appearance index increased from 1.8 to 4.7 and the average elasticity index from 1.1 to 3.8. The analysis of the comparisons between the different phases of therapy have showed that each subsequent treatment determined a significant increase in all parameters, except for the vaginal elasticity and the vaginal wall epithelium appearance for the

Table I. VAS scale for the intensity of the three following symptoms.

Symptoms	
Score 1	Maximum intensity
Score 2	Strong intensity
Score 3	Average intensity
Score 4	Mild intensity
Score 5	Weak intensity
Score 6	Absence of symptom

(1) well-being such as absence of dyspareunia, vaginal dryness, vulvar and/or vaginal itching, (2) vaginal burning (3) vaginal lubrication and presence of fluid.

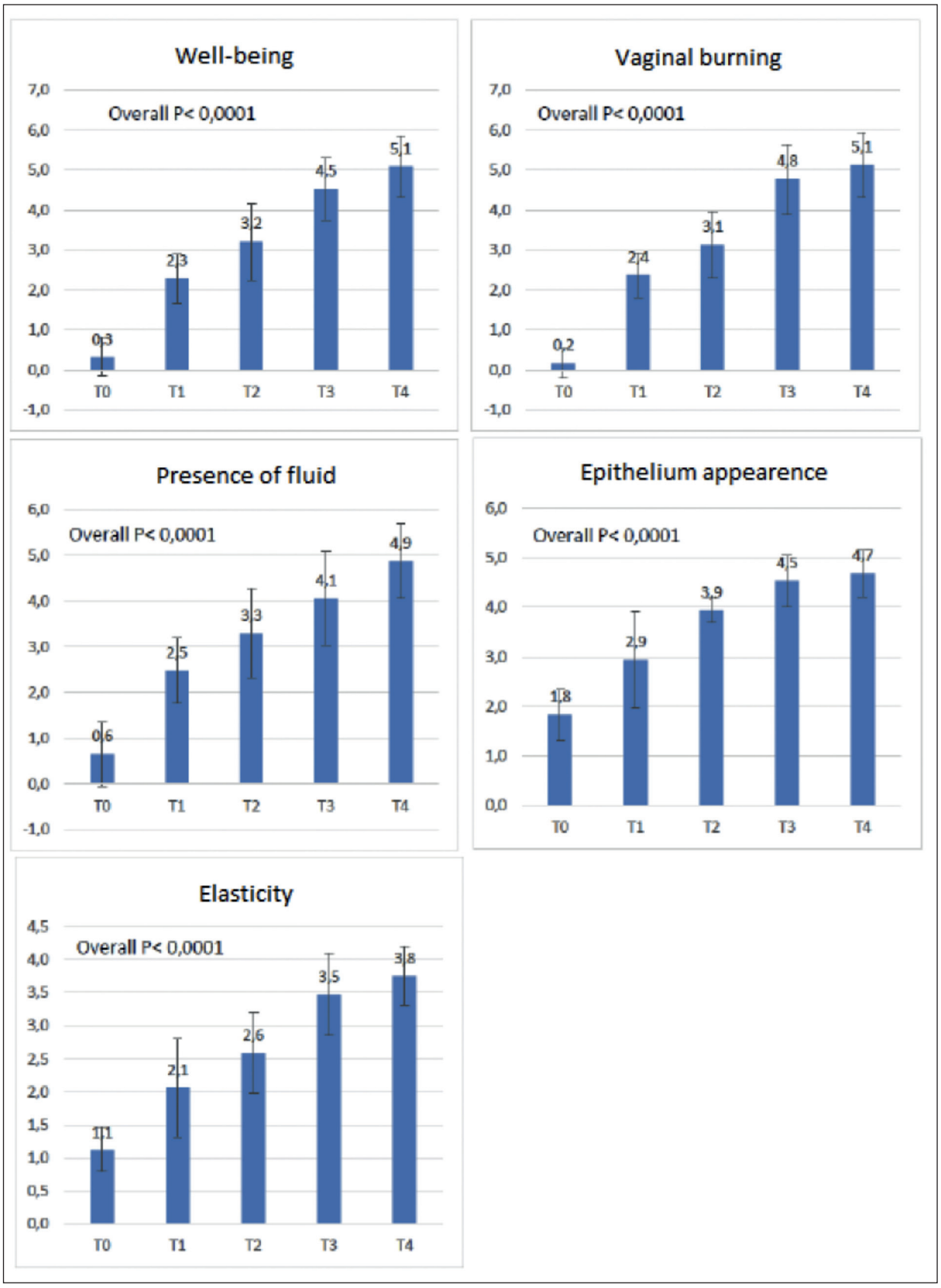

Figure 1. Trend of variation of mean score values between treatment sessions.

Table II. Vaginal elasticity and vaginal wall epithelium appearance determined with two numerical scores.

Vaginal elasticity		Vaginal wall epithelium appearance	
Score 1	Absent	Score 1	Petechiae
Score 2	Poor	Score 2	Contact bleeding
Score 3	Average	Score 3	Scratching bleeding
Score 4	Good	Score 4	Erythema
Score 5	Excellent	Score 5	Normal

last treatment (interval T3-T4) (Table III). All the patients reported a recovery of their sexuality at the end of the five treatment sessions.

Discussion

Despite the high prevalence and the substantial effect on quality of life, VVA often remains underestimated and not subjected to treatment[13]. LET is the effective standard therapy, but many women in this condition refuse its use because of negative publicity in recent years related to the side effects. In fact, while less recent studies[14-17] showed an increased breast cancer risk with estrogen and progestin combination, which has led to the perception of estrogen as a harmful treatment, later publications showed that the risk has been overestimated and limited to selected types of combinations, whereas estrogens alone seem to be protective instead[15-17]. Furthermore, there are few safety studies[18] supporting the use of LET in breast cancer survivors, and this therapy is considered contraindicated. The SERM ospemifene is currently indicated in Europe for the "Treatment of moderate to severe symptomatic VVA in post-menopausal women who are not candidates for LET". Although women with contra-indications to LET are clearly 'not candidates for LET'. It is ultimately at the discretion of the treating physician whether to prescribe ospemifene in that case or not[19,20]. Patients who have survived breast cancer and have completed their adjuvant treatment are not candidates for LET due their history of breast cancer but can use ospemifene[19]. Patients who have not completed follow-up or who are performing adjuvant therapy can only use the non-hormonal vaginal moisturizers, lubricants designed to treat VVA symptoms. These treatments address vaginal dryness and reduce burning dyspareunia and hitching, but they have no effect on the loss of elasticity and compliance of vaginal walls, and their effect is only transitory. A recent report[21] by the North American Menopause Society has not taken a position on how to treat women with early menopause in cases of breast, ovarian or endometrial cancer, suggesting that the management of the problem should be left to the oncologist, considering the potential risk of hormone treatment in these subjects. In this context the therapy with hyperbaric oxygen in combination with hyaluronic acid could be proposed in the future to the patients. Oxygen therapy determines the increase of the reparative processes of the tissues and increases the synthesis of collagen, allowing a normal hydroxylation of this protein[22]. In fact, at oxygen tensions lower than normal, the collagen is not correctly synthesized, delaying the healing of the wounds. Furthermore, oxygen induces a neo-angiogenic stimulation through the release of VEGF[23]. This function is essential for the restoration of the microcirculation in compromised vascular tissues, re-establishing a vascular flow in the hypoxic areas[24]. Hyaluronic acid is a natural polysaccharide, which is an important part

Table III. Comparisons between the different phases of therapy in relation to the intensity of the three following symptoms and of the vaginal elasticity and the vaginal wall epithelium appearance

	T0-T1	T0-T2	T0-T3	T0-T4	T1-T2	T1-T3	T1-T4	T2-T3	T2-T4	T3-T4
Well-being	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$
Vaginal burning	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.05$
Presence of fluid	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.05$
Epithelium appearance	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	NS
Elasticity	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NS

of the extracellular matrix of skin and cartilage. This substance can bind a large quantity of water molecules contributing to the maintenance of water balance, proper hydration and structure of skin and mucous membranes[25]. Various researches[26,27] of hyaluronic acid in VVA therapy have shown that this compound has been well tolerated without side effects. Our pilot study has shown very high efficacy of the VNOD, both in the analysis of subjective and objective data. The data showed a reduction of burning symptoms already after the first session, reaching a value of 5.1 (on a scale of 6) at the end of the therapy. One of the most immediate effects was the increased elasticity of the tissues, already after the first session, reaching a value of 3.8 (on a scale of 5) at the end of the therapy. All the indexes used show a statistically significant improvement at each treatment except for the epithelium and elasticity parameters in the T4-T5 (although the data showed an improvement), suggesting that for these scores, 4 sessions are sufficient to achieve the maximum result with this therapy. VVA is often a cause of urinary tract disorders. Some patients reported an improvement of the genitourinary symptoms such dysuria, pollakiuria and mild incontinence. Our pilot study was not aimed at the evaluation of these disorders. Further studies could confirm the effect on urinary symptoms. Moreover, to demonstrate the effectiveness of this combined therapy, a double-blind, randomized, controlled, trial is required. This study will be completed with the analysis of the degree of restoration of the mucosa (epithelium and connective tissues) by histological and immunohistochemical analyses. The role exerted by Mesenchymal Stem Cells (MSCs) in the reparative process will also be studied.

Conclusions

We observed that the use of VNOD has proven to be a valid treatment of VVA, resulting in a completely natural type of therapy well accepted by patients with immediate therapeutic effects and without side effects. A following larger pivotal trial may support such data and study other beneficial effects of such therapy.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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