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Original Research

Efficacy of an oral supplement containing hyaluronic acid, collagen, glucosamine sulfate, chondroitin sulfate, alpha-lipoic acid, methylsulfonylmethane and vitamins on vaginal dryness in young women

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Abstract

This study evaluated the oral administration of a new supplement containing a combination of bioactive agents (hyaluronic acid, glucosamine sulfate, chondroitin sulfate, alpha-lipoic acid, methylsulfonylmethane and vitamins A, C and E).

The objective of the study was to demonstrate that the oral administration of this product combats vulvovaginal atrophy in young women.

This observational study recruited 32 women of fertile age with vaginal dryness, post-coital cystitis and/or dyspareunia (painful intercourse).

Patients were evaluated using the VHI scale and the FSFI at baseline and at each follow-up. All patients took one tablet of the supplement twice daily (morning and evening) for two months.

After two months, 28 patients (87%) showed an improvement in symptoms and reported complete remission.

At the first follow-up at three months, that is, one month after stopping treatment, no substantial changes were reported. At the second follow-up at four months, that is, two months after stopping treatment, vaginal dryness had recurred, and patients were asked to re-start the therapy.

We can conclude that this product is highly effective in young women suffering from vaginal dryness and sexual dysfunction caused by oxidative stress.

Keywords: Hyaluronic acid, nutraceuticals, vaginal dryness

Introduction

Vaginal dryness, dyspareunia and sexual disorders are an important problem for postmenopausal women and numerous pharmaceutical products and medical devices have been created to treat these [1]. Unfortunately, women of reproductive age cannot always use the same products or devices as postmenopausal women. In addition, no clear guidelines exist for the management of sexual dysfunction in women of reproductive age.

Vaginal dryness in young women can be caused by oral contraceptives, other medicinal products (psychotropic drugs), smoking, altered microbiota, excessive hygiene and postpartum and psychological disorders (anxiety, depression, stress) [2–5]. Local vaginal lubricants containing vitamin A or E, vegetable oil, herbal supplements and/or hyaluronic acid may provide temporary relief from the discomfort of vaginal dryness associated with sexual activity, but they are palliative treatments that require continuity in their application. Long-term adherence is very poor, and vulvar discomfort may lead to psychological trauma [1]. Recent publications have reported excellent results on dermal function with orally administered biologically active compounds (such as antioxidants), leading to the development of nutritional supplements for damaged human skin [6–8]. The oral administration of hyaluronic acid, collagen and other antioxidant compounds has also proved to be very effective against osteoarthritis and articular cartilage disorders [9, 10].

This study evaluated the oral administration of a new supplement containing a combination of bioactive agents. This nutricosmeceutical contains a European-patented complex containing the bioactive ingredients hyaluronic acid, glucosamine sulfate, chondroitin sulfate, alpha-lipoic acid, methylsulfonylmethane and vitamins A, C and E. The objective of the study was to demonstrate that the oral administration of Unilen Hydravit® (UH) combats vulvovaginal atrophy in young women.

Materials and methods

This observational study recruited 32 women of fertile age with vaginal dryness, post-coital cystitis and/or dyspareunia (painful intercourse) in the previous 24 months.
The study was conducted by a private OB-GYN practice in Rome, Italy. All patients provided their written informed consent prior to participation in the study. All patients underwent a clinical evaluation prior to enrolment in the study. All patients suffered from vaginal dryness and dyspareunia, while three also reported post-coital cystitis.

The Vaginal Health Index (VHI) score, which investigates vaginal elasticity, fluid volume, pH, epithelial integrity and moisture on a scale of 1 to 5, with a minimum overall score of 5 and a maximum overall score of 25 [11], was administered at baseline, at the end of treatment (two months), and at the three- and four-month follow-ups. All patients had a VHI score between 13 to 25 at baseline. In addition, the Female Sexual Function Index (FSFI) [12] was administered at baseline and at each follow-up to measure the improvement in sexual response after treatment with the product. This index investigates lubrication, arousal, satisfaction, sexual desire and improvement in orgasmic ability.

The inclusion criteria were a negative urine culture, Pap smear and HPV DNA detection, and a negative high vaginal swab for *Candida albicans*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. Patients who had taken antibiotics for their post-coital cystitis and/or antimicrobial topical agents for vaginal infections in the previous month, or who had vulvovaginal atrophy caused by the menopause, were

excluded from the study. pH indicator paper was used to determine the pH of the vaginal discharge. All patients took one tablet of the supplement twice daily (morning and evening) for two months. The name of the supplement is Unilen Hydravit® and it is manufactured by Uniderm (Rome, Italy).

Results

Methodology

Questionnaire data for each patient were reported in a Microsoft Excel file (Microsoft Corp., WA, USA) and analysed using Stata15 (StataCorp., TX, USA). Percentages and frequencies were used for descriptive statistics of qualitative data, while means and standard deviations were used for age and VHI and FSFI scores. Comparisons were performed using the Wilcoxon matched-pairs signed-ranks test, given the non-normal distribution of data.

The statistical significance was set at the level of 5% (p<0.05). Statistical analysis was performed on VHI and FSFI scores with attention focused on four stages: baseline (T0, beginning of treatment); two months from the beginning of treatment, coincident with treatment end (T1); three months later (one month after treatment suspension, T2); and four months later (two months after treatment suspension, T3).

Data analysis

Thirty-two patients were enrolled in the study and completed the treatment protocol. The mean age of the patients was 35.3 ± 7.20 years. 56.3% of the sample was composed of patients with a university degree, followed by 34.4% with a high-school diploma. Twenty-five patients (78%) lived in the city (Rome) and the remainder in the countryside. 43.8% of patients were smokers (10–20 cigarettes per day), while 50% of the sample worked out in the gym regularly or played a sport. 40.6% of patients used oral contraceptives. 31.3% of the sample was affected by a disease: one patient (3.1%) had coeliac disease, one (3.1%) had psoriasis, three (9.4%) had recurrent cystitis, three (9.4%) had PCOS, one (3.1%) had vitiligo and one (3.1%) had gastroesophageal reflux disease (**Table 1**).

N. Patients, obs.	32
Age, mean (s.d.)	35.3 (7.2)
Education (%)	
Junior High School	3 (9.4)
High School	11 (34.4)
Degree	18 (56.3)
Smoking (%)	14 (43.8)
Physical activity (%)	16 (50.0)
Oral contraceptives (%)	13 (40.6)
Disease (%)	10 (31.3)
Coeliac disease	1 (3.1)
Psoriasis	1 (3.1)
Recurrent cystitis	3 (9.4)
PCOS	3 (9.4)
Vitiligo	1 (3.1)
Gastroesophageal reflux	1 (3.1)

Table 1 Patient characteristics

At baseline, all patients presented an atrophic vagina (minimum VHI total score was 13 points). Overall, after two months and at the end of treatment, 28 patients (87%) showed an improvement in symptoms and reported complete remission, as documented by the increase in both FSFI and VHI total scores (**Fig. 1**). More specifically, there was a significant difference between T1 and T0 (p<0.01) with an increase in both the VHI total score (T0: 13.19 ± 1.38; T1: 24.19 ± 0.78) and the FSFI total score (T0: 12.24 ± 1.50; T1: 18.41 ± 0.62). This significant difference was also apparent in the ensuing follow-ups: comparing T0 and T2, total scores of VHI (T2: 21.47 ± 1.05) and FSFI (T2: 17.18 ± 0.82)

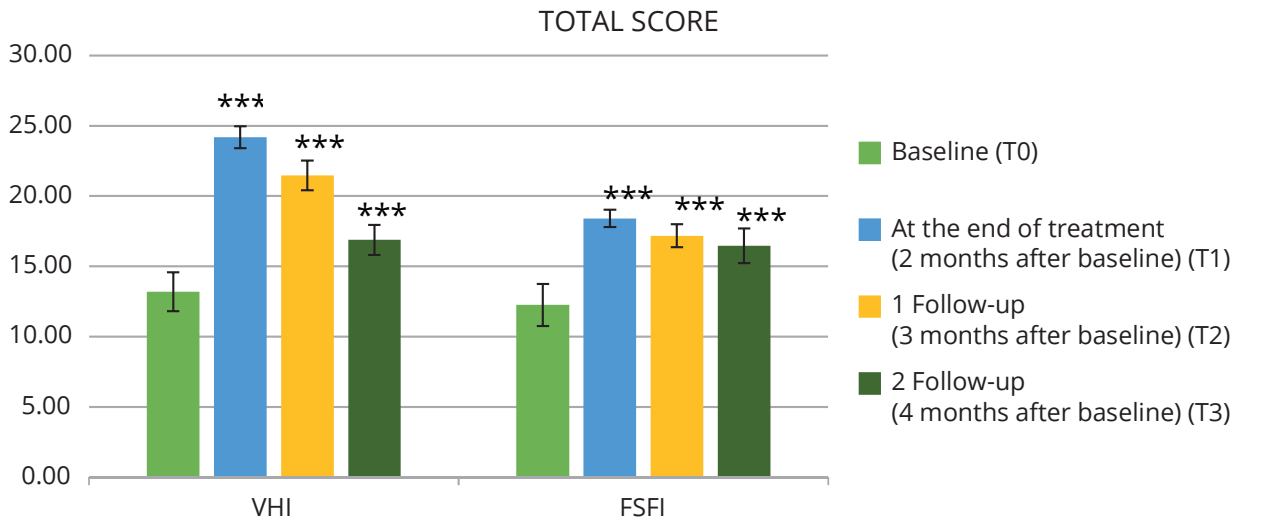

Figure 1 Total scores for the Vaginal Health Index (VHI) and the Female Sexual Function Index (FSFI). After two months and at the end of treatment, 28 patients (87%) showed an improvement in symptoms and reported complete remission, as documented by the increase in both FSFI and VHI total scores. ***p<0.01 compared to baseline (T0)

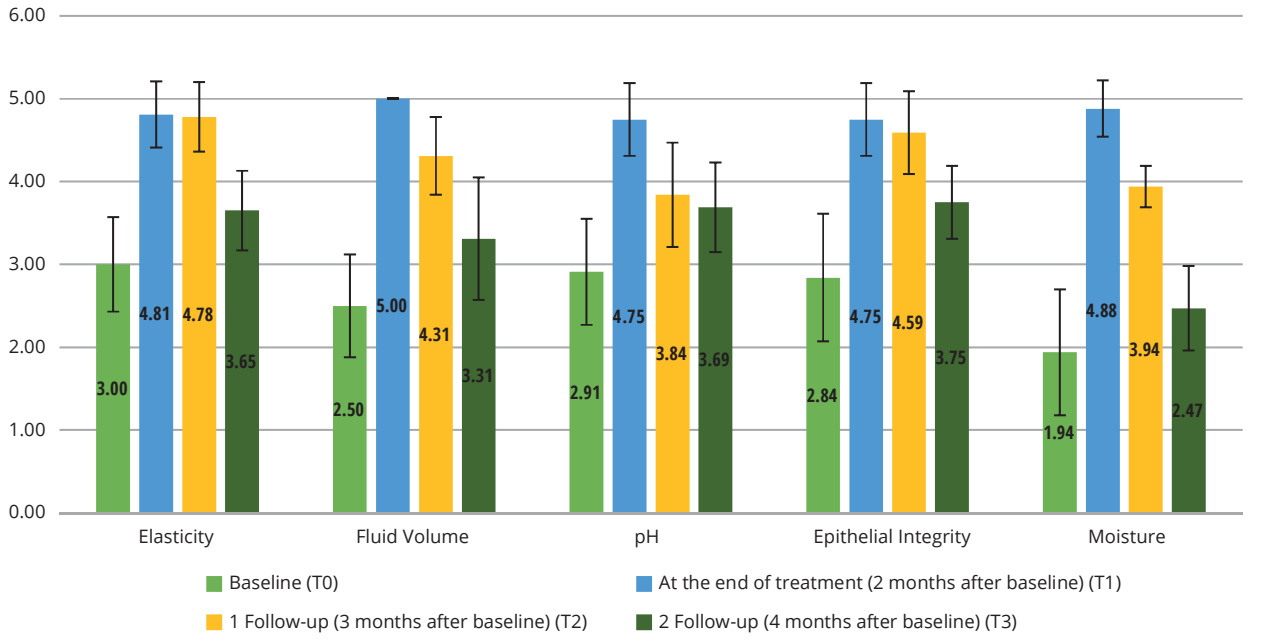

Figure 2 Vaginal Health Index (VHI) score per single domain. Comparing values at baseline (T0) with those obtained at the end of treatment (T1) and at the two follow-ups after treatment suspension (T2 and T3), an improvement in all of the individual domains of the VHI was observed (n=28). Each comparison between baseline and the three later measurements was characterized by a significant difference of p<0.01

remained significantly higher (p<0.01) than T0. A significance level of 1% was also noted with regard to the comparison between T0 and T3, with both of the total scores at T3 higher than baseline values (VHI T3: 16.88 ± 1.07; FSFI T3: 16.46 ± 1.23).

VHI Scores

Comparing values at baseline (T0) with those obtained at the end of treatment (T1) and at the two follow-ups after treatment suspension (T2 and T3), an improvement in all of the individual domains of the VHI score was observed (**Fig. 2**): each comparison between the baseline and the three later measurements was characterized by a significant difference at a level of 1% (p<0.01). Regarding the comparison between T1 and the two follow-ups, there were different results for some domains. Specifically, the mean value at

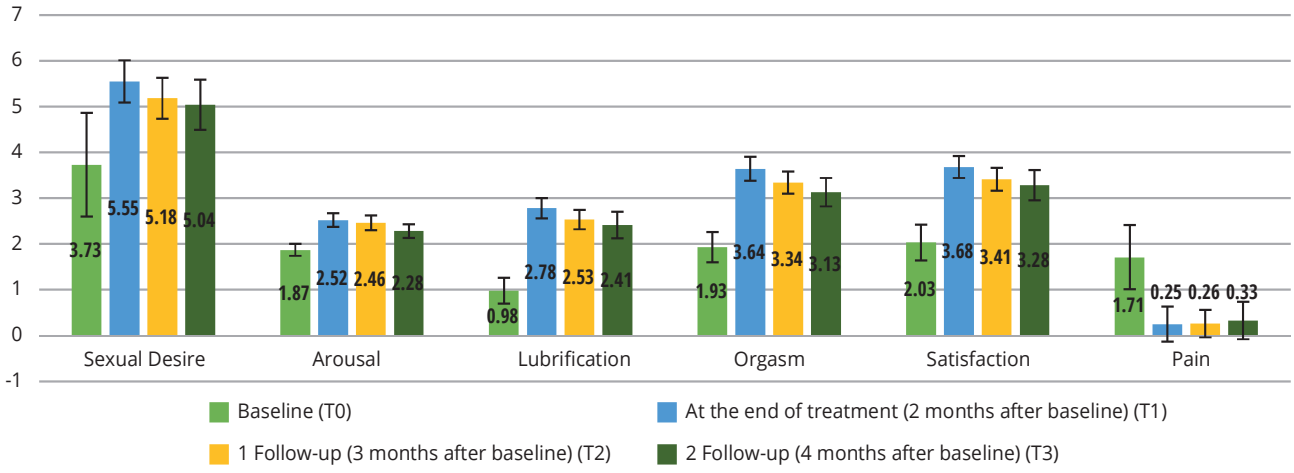

Figure 3 Female Sexual Function Index (FSFI) score per single domain. An improvement was observed from the baseline to T1 for sexual desire, arousal, lubrication, orgasm and satisfaction (p<0.01). For pain, a significant decrease in the mean value was seen from baseline to the end of treatment, and this reduction in pain values persisted for the two subsequent follow-ups

T1 (4.81 ± 0.40) for elasticity was higher than that registered at T2 (4.78 ± 0.42), without any statistical significance (p>0.05). Comparing the T1 mean value with that achieved at T3 (3.65 ± 0.48), the value at T1 was higher than that at T3 and this difference was characterized by statistical significance (p<0.01). The decrease observed at T3 was also significant in comparison with T2 (p<0.01). Regarding fluid volume, there were differences between T1 (5.00 ± 0.0) and T2 (4.31 ± 0.47) and T1 and T3 (3.31 ± 0.74) (both p<0.01). The same trend was seen when comparing T2 and T3, where the T2 value was higher than that registered at T3 (p<0.01). The pH mean value registered at T1 (4.75 ± 0.44) was higher than those recorded at T2 (3.84 ± 0.63) and T3 (3.69 ± 0.54) (both p<0.01), and the difference between T2 and T3 was also significant (p<0.05), although at a lower level.

For epithelial integrity, there was a significant difference (p<0.01) between T1 (4.75 ± 0.44) and T3 (3.75 ± 0.44), but not between T1 and T2 (4.59 ± 0.50), while the comparison between T2 and T3 was statistically significant (p<0.01). Finally, regarding the last domain of the VHI – moisture – there were significant differences between T1 (4.88 ± 0.34) and T2 (3.94 ± 0.25), T1 and T3 (2.47 ± 0.51) and T2 and T3 (all p<0.01).

FSFI scores

With respect to the FSFI scores, an improvement was observed between the baseline and T1 in terms of sexual desire, arousal, lubrication, orgasm and satisfaction, as seen in **Fig. 3**. For pain, there was a significant decrease in the mean value from baseline to the end of treatment, and this reduction in pain values persisted for the two subsequent follow-ups.

Comparing the mean values of the sexual desire domain at T1 with those recorded at T2 and T3, there was a decrease from T1 (5.55 ± 0.46) to T2 (5.18 ± 0.45) and T3 (5.04 ± 0.55). All differences were significant (p<0.01). Regarding arousal, a T1 mean value of 2.52 ± 0.15 was observed, while the T2 and T3 mean values for arousal were lower (T2: 2.46 ± 0.16; T3: 2.28 ± 0.15). Both comparisons between T1 and T2, and T1 and T3 were significant (p<0.01). The difference in the arousal mean values of T2 and T3 was also statistically significant (p<0.01). The lubrication mean value was higher at T1 (2.78 ± 0.22) than at T2 (2.53 ± 0.21) and at T3 (2.41 ± 0.29). In this case, all differences were significant at a level of 1%. For the orgasm domain, the mean value at T1 (3.64 ± 0.26) was higher than that recorded at T2 (3.34 ± 0.24) and at T3 (3.13 ± 0.31), both p<0.01. Between the orgasm mean value at T2 and that at T3,

there was also a significant difference (p<0.01). The same trends were seen with respect to satisfaction: the mean value at T1 (3.68 ± 0.24) was higher than that recorded at T2 (3.41 ± 0.25) and at T3 (3.28 ± 0.33), and the mean value at T2 was higher than that at T3.

All differences were characterized by a statistical significance (p<0.01). Finally, for pain, there was a reduction in the mean value, and there were no statistically significant differences between T1 and T2, T1 and T3 and T2 and T3.

Discussion

UH is a nutricosmeceutical that contains several bioactive ingredients acting on the skin and mucous membranes. Hyaluronic acid (HA) is a glycosaminoglycan consisting of repeating D-glucuronic acid and N-acetyl-D-glucosamine disaccharide units. It is ubiquitously distributed in the extracellular matrix and is found at high concentrations in the connective tissue, synovial fluid, vitreous humour, umbilical cord and skin. It has a molecular weight of more than 1 MDa. It is water-retentive and can bind with water approximately 1000-fold its own weight [13]. HA has several physiological functions, including cell maintenance, water preservation and nutrient transport [14]. It is also involved in mediating other biological functions and receptors (such as the interaction between CD44 and toll-like receptors), including anti-inflammatory actions and bone morphogenetic effects [15–17].

Glucosamine sulfate and chondroitin sulfate are complex sugars. Glucosamine sulfate is a common constituent of cartilage and synovial fluid, and exerts various pharmacological effects on the articular cartilage and joint tissue [18]. Chondroitin sulfate, a major component of the extracellular matrix, is a sulfated glycosaminoglycan responsible for many of the important biomechanical properties of cartilage, such as resistance and elasticity [19].

Alpha-lipoic acid (ALA) is a potent antioxidant and is involved in energy metabolism.
Its main biological role is as a cofactor to mitochondrial enzymes, such as alpha-ketoglutarate dehydrogenase and pyruvate dehydrogenase. It also appears to be involved in the production of acetyl-CoA, via oxidative decarboxylation of pyruvate. ALA supplementation has been found to provide protective benefits against oxidation, inflammation, diabetes and cognitive decline [20, 21]. Methylsulfonylmethane (MSM), also known as methyl sulfone or dimethylsulfone, is the first oxidized metabolite of dimethyl sulfoxide, a strong solvent used in the treatment of interstitial cystitis [22].
MSM is used in skin care, for the treatment of osteoarthritis pain, to improve digestive function and to catalyse the healing process [23].

Vitamin A is a fat-soluble vitamin with an important antioxidant role, reducing inflammation by combating free radical damage.
It is essential in maintaining healthy vision, neurological function and skin [24]. Vitamin C, also known as ascorbic acid, is a water-soluble vitamin found in many types of fruit and vegetables. It acts as an antioxidant to neutralize free radicals and to reduce the risk of inflammation and disease. It is essential for the synthesis of important compounds such as collagen, the structural protein found in connective tissue, and contributes to wound healing [25].

Alpha-tocotrienol and gamma-tocotrienol are isomers of vitamin E. They too can reduce damage from free radicals and inflammation [26] and can significantly increase immunity [27]. Vitamin E also improves skin moisture and elasticity, acting as an antioxidant against damage from smoking or ultraviolet light [28].

There is considerable evidence in the literature of the benefits of hydrolyzed collagen-based nutraceutical supplements on human skin [29–34]. Some dietary components with antioxidant properties could have an indirect effect on the skin through secondary messen-

gers. Following ingestion, they can pass down the gastrointestinal tract, cross the intestinal barrier, and enter the circulation, thus being delivered to various body tissues, including the skin. The blood can continuously replenish the skin with these bioactive compounds, which are then distributed to all skin compartments (epidermis, dermis, subcutaneous fat) [35].

The absorption and distribution of hydrolyzed collagen has been analyzed in several different studies [36, 37]. It has also been shown that collagen-derived peptides can be absorbed through the PEPT1 transporter (located in the intestinal brush border membrane) [38].

In contrast, the topical application of products containing collagen or hyaluronic acid does not significantly improve the skin texture, because the collagen molecules cannot penetrate the epidermis, due to their high molecular weight (130–300 kDa) [39]. The low molecular weight of collagen bioactive peptides and/or hyaluronic acid permits their systemic absorption and distribution to the various tissues following oral administration [36, 37].

The vulva is composed of multiple anatomic structures, which have distinctive characteristics. Some areas such as the mons pubis and the labia majora consist of hair-bearing skin and abundant richly innervated and vascularized subcutaneous tissue. The labia minora are characterized by keratinizing squamous epithelium without skin adnexal structures.

The vulvar vestibule, surrounding the vaginal introitus, is the border between dermal tissue and mucosal tissue, and hence, between the vulva and vagina. The vaginal mucosa extends from the vestibule to the cervix and is lined by stratified, non-keratinizing, squamous epithelium [40]. It is clear that the vulva comprises both dermal tissue and mucosa and a variety of solutions have therefore been developed to improve and maintain vulvovaginal health: local preparations (creams, tablets, gels, rings) [41], vaginal electroporation [42] to enhance drug diffusion for transmucosal delivery of macromolecules and vaginal laser therapy [43–45] to treat symptoms of vulvovaginal atrophy. Local vaginal lubricants containing vitamins A or E, vegetable oil, herbal supplements and/or hyaluronic acid may provide temporary relief from the discomfort of vaginal dryness associated with sexual activity, but they are palliative treatments that require continuity in their application. Long-term adherence is very poor, and vulvar discomfort can lead to psychological trauma [41].

Several studies have associated the use of oral combined hormonal contraception (CHC) with adverse effects on sexual function, particularly sexual desire [2]. This occurs because oral CHC increases sex hormone-binding globulin (SHBG), reduces free testosterone and stops ovarian androgen production [46]. The outcome is a reduction in circulating androgen levels, which can determine the loss of collagen and water-retaining capacity from the vulva and vagina. This results in the onset of vaginal dryness and reduction in lubrication, arousal and sexual pleasure [47]. Although oral CHC may be a contributing factor, it is also useful to review the patient's medical history (for example, a history of pelvic surgery, diabetes mellitus and cardiovascular or neurological disease) and any other medications that might exacerbate discomfort (for example, antidepressants, opioid pain medications and some antihypertensive medications).

Nicotine and its metabolite cotinine have been detected in the cervical mucus of smokers [48–50] and an accumulation of vaginal amines is conceivable. Traces of benzopyrene diol epoxide (BPDE) are found in the vaginal secretions of women who smoke and BPDE significantly increases bacteriophages, reducing lactobacilli protection [51]. The anti-oestrogenic effect of smoking predisposes woman to bacterial vaginosis, and subsequently, to vaginal discomfort [52]. Stress, anxiety, depression and substance abuse can all play an important role

in this problem. Social and religious convictions and inadequate sex education can also contribute to sexual dysfunction. Consultation with a sex therapist may be helpful for women with sexual pain or with low sexual desire [2].

Hormonal contraception, smoking, antidepressants and over-frequent washing with harsh cleansers all create oxidative stress within the vulvovaginal tissues. Oxidative stress generates reactive oxygen species (ROS) which are ultimately responsible for damage to cells, enzymes, membrane lipids and not least to the extracellular matrix, rich in collagen and hyaluronic acid, resulting in the need for supplementation with these components. Our study showed that oral supplementation with hyaluronic acid, collagen, glucosamine sulfate, chondroitin sulfate, alpha-lipoic acid, methylsulfonylmethane and vitamins A, E and C restores vulvovaginal trophism. After two months, 28 patients (87%) showed an improvement in symptoms and reported complete remission, as documented by an increase in both FSFI and VHI scores.

Two patients had complained at baseline of perineal tearing on sexual intercourse with pain and bleeding; this had completely resolved after treatment. No discomfort or adverse reactions were reported. Neither patient used local vaginal therapy. During the second month of follow-up, perineal tearing recurred in one of these patients. The three women with recurring bouts of cystitis, especially post-coital, did not report any episodes during the treatment, and no recurrent episodes of cystitis were reported after its cessation. This result confirms the findings of a previous study by Torella et al [53], in which the orally administered combination of hyaluronic acid, chondroitin sulfate, curcumin and quercetin was effective in preventing recurrent urinary tract infections. No discomfort or adverse reactions were reported in our study. During the two months of follow-up the benefits deriving from supplementation with UH were maintained.
Two months after the end of therapy vaginal dryness recurred, and 78% of all patients asked to re-start the treatment. We therefore conclude that oral supplementation with UH is highly effective in young women suffering from vaginal dryness and sexual dysfunction caused by oxidative stress. Randomized multicentre studies are needed to establish a standard therapy for young women suffering from vulvovaginal discomfort and/or recurrent post-coital cystitis.

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