GENITOURINARY SYNDROME OF MENOPAUSE AND THE ROLE OF BIOSTIMULATION WITH NON-CROSS-LINKED INJECTABLE HYALURONIC ACID PLUS CALCIUM HYDROXYAPATITE

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To the Editor,

Genitourinary Syndrome of Menopause (GMS) (1) includes a wide range of urogynaecology symptoms, such as dryness, irritation, itching, burning and dyspareunia, and is caused by estrogen depletion associated with the aging process. In GMS, the progression of vulvovaginal atrophy (VVA) ends with either hyalinization of connective tissues or loss of vaginal elasticity and function and consequent dryness that leads to dyspareunia and a reduction of sexual activity (2).

Even if the mechanisms involved are multi-factorial, it has been observed that stress urinary incontinence (SUI) is related to menopause due to a decrease in collagen and modification of its composition. Pharmacological options in the restoration of connective tissue aging-dependent changes can be preventive, restitutive, curative and palliative. Recitative treatments are defined as procedures aimed at restoring metabolism and function of connective tissue, the most important of which is bio-stimulation activating fibroblast anabolic functions, and particularly enhancing type III collagen, elastin and hyaluronic acid production from their precursors. Thus, in urogenital functional restoration, bio-stimulation could be considered as an innovative approach according to the principles of antiaging medicine. However, few studies support hyaluronic acid (HA) application during a simple outpatient procedure as possible therapeutic strategy (3). HA has a low potential risk for immunogenic reactions and shows no tissue or species antigenicity.

The aim of this double-blind randomized controlled clinical and histological study is to evaluate the efficacy of a treatment protocol based on the injection of a HA plus calcium hydroxyapatite in vaginal walls, based on filler propriety to activate collagen and promote elastin restoration at molecular level, improving vaginal epithelium thickness, elasticity, lubrication and function such as secretion and absorption.

Key words: genitourinary; menopause; vulvovaginal atrophy

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MATERIALS AND METHODS

The goal of this study was to evaluate the efficacy of a treatment protocol based on the injection of a non-cross linked hyaluronic acid (at a concentration of18 mg/ml) plus calcium hydroxyapatite in the lateral vaginal walls to activate collagen and promote elastin restoration at a molecular level, improving vaginal functions such as secretion, absorption, elasticity, lubrication and epithelium thickness, achieving a reduction in the symptoms related to the GSM, with no complications, side effects, local reactions or systemic complications for the patient.

A double-blind randomized controlled clinical trial was conducted from October 2017 to March 2018 at the Urogynecology Unit, San Jorge University Hospital, Pereira, Colombia. This study included 20 volunteer postmenopausal women who presented genitourinary symptoms related to GSM in the previous 24 months, such as SUI or mixed urinary incontinence detected by the International Consultation on Incontinence Questionnaire-Urinary Incontinence Short Form (ICIQ UI SF), or vaginal symptoms evaluated by the Vaginal Health Index (VHI) (4). Sexual function was evaluated using the Female Sexual Function Index (FSFI) score.

Exclusion criteria were patients not adequately classified, previous gynecological surgery, recurrent lower urinary tract infection, BMI > 35, cognitive or psychiatric disorders, pelvic floor dysfunction and pelvic organ prolapse > 2 according to the Pelvic Organ Prolapse Quantification System (POP-Q).

The demographic and clinical data of the study population are shown in Table I. All patients included in the study provided written informed consent and the procedure was compliant with the principles of the declaration of Helsinki, Belmont Report, Council for International Organizations of Medical Sciences rules, GPC/ICH and 008430 resolution of the Colombian government established on 4th October, 1993.

The 20 volunteer patients enrolled in this study were randomized prospectively in a 3-month follow-up into two groups:

- 10 patients in the Treatment Group (TG), were injected with not cross-linked hyaluronic acid (NCLHA) plus calcium hydroxyapatite in vaginal walls.
- 10 patients in the Control Group (CG) were injected with saline solution in vaginal walls.

The product is a sterile, pyrogen-free gel of NCLH, containing 18 mg/ml of pure HA, enriched with 0.01% of calcium hydroxyapatite (CaHA) plus Glycine and L Proline (Neauvia Hydro Deluxe, Matex Lab SA, Switzerland). For the randomization process, patients with similar urogenital symptoms were allocated into the two groups; the study was performed by two investigators and the treatment protocol was performed by the main investigator. The injection was performed in the lateral vagina wall, around the urethra-vesical junction, with a 27G/37mm disposable blunt microcannula. The procedure was made through the fan technique, and a quantity of 2 ml of the product was injected on each side.

Vaginal biopsies were obtained before the injections and after 3 months. The vaginal samples were sent for basic and special histological studies performed by a blinded pathologist in order to demonstrate vulvar and vaginal trophic changes after the treatment protocol. Two kinds of stains were used**,** Haematoxylin and Eosin (H&E**)** and modified Masson's trichrome. With the H&E, the nucleus and parts of the cytoplasm that contained ribonucleic acid were stained blue, while the rest of the cytoplasm were stained in pink because most of the proteins in the cytoplasm are basic, and eosin binds to those proteins thus staining them in pink. In wound healing, collagen fibers play a dominant role in maintaining structural integrity. The Modified Masson's trichrome was modified according to Kiernan. Stains were primarily used to show collagen and muscle in normal tissue, this stain is also used to distinguish collagen from muscle, to demonstrate an increased collagen deposition in tissue or to indicate fibrotic changes.

The primary endpoint of this study was a clinical assessment of changes related to vaginal tropism. The evaluation was made through clinical examination, change of scores in the VHI, and FSFI referred to desire, arousal, lubrication, orgasm and satisfaction. The secondary endpoint of the study was to assess the reduction of the symptoms related to the GSM, through the evaluation of ICIQ UI SF and the treatment satisfaction score after that was evaluated with a visual analog scale (VAS) from baseline conditions to the end of the treatment protocol.

Statistical analysis was performed using SPSS 11.5.1 software (SPSS, Chicago, Illinois. USA). The following quantitative variables were evaluated: mean, median,

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and standard deviation in both groups. *Chi*-squared and Fisher's exact test were used to evaluate categorical variables of patients between the two groups (education level, marital status and parity).

RESULTS

The two groups were considered homogenous regardless of socio-demographic and clinical

Table I. *Demographic and clinical data of the patients.*

	Control Group (CG)n=10	**Treatment Group (CG)n=10**
Age (Years) SD	58.1 (±3.77)	57.9 (±3.22)
BMI (Kg/m2) SD	28.05 (±1.27)	28.35 (±1.55)
Parity SD	3.0 (±0,8)	2.7 (±0.8)
Marital Status %		
Married	60.0	50.0
Single	40.0	50.0
Education Degree %		
Illiterate	0	0
High School	67.3	60.3
University	32.7	39.7
CLINICAL CHARACTERISTICS		
Anterior Pelvic Organ Prolapse %		
Stage 0	0	0
Stage I	82.31	17.69
Stage II	42.33	57.67
Stage III	0	0
Mode of Delivery %		
Vaginal	40.7	59.3
Forceps	3.12	4.10
Cesarean Section	56.18	36.6
Urinary Incontinence		
Presence	83.33	83.33
No presence	16.7	16.7

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Table II. *FSFI score at baseline and 12 weeks after the injection of NCLHA plus calcium hydroxyapatite (TG) or saline solution (CG), expressed as mean value SD.*

FSFI Domains	Desire	Arousal	Lubrication	Orgasm	Satisfaction	Pain
Baseline TG	2.5±1.2	3.1±1.6	3.8±1.7	3.5±1.6	4.4±1.3	3.8±1.9
12-Week Follow up TG	3.4±1.1	3.9±1.3	4.3±1.7	4.2±1.6	4.7±1.6	4.7±1.7
p	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)	(p<0.01)
Baseline CG	2.6±0.9	2.1±0.7	1.2±0.4	1.8±0.4	2.8±0.4	3±0.4
12-Week Follow up CG	2.4±0.6	2.3±0.6	1.2±0.4	1.8±0.4	2.7±0.4	2.7±0.4

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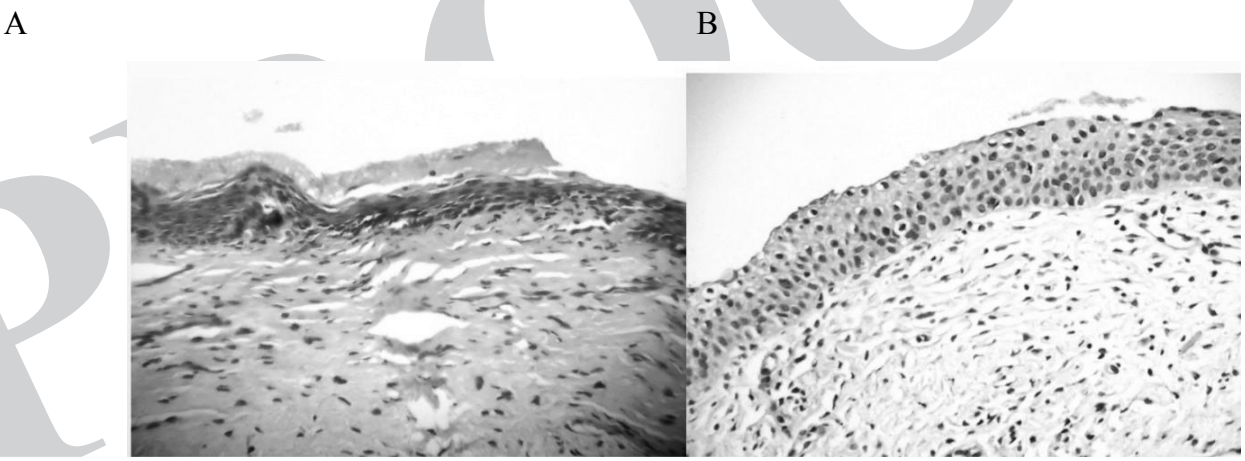

Fig. 1. *(H&E stain). Vaginal sample. Pre-treatment (**A**) and 3 months after the injection of NCLHA + CaHa (**B**). Before the treatment the epithelium of the vagina appeared flattened, atrophic, with few cell layers, small cells and dense stroma, while 3 months after the treatment with NCLHA plus CaHa, the epithelium of the vagina appeared thicker, with many cell layers, with stroma growth, enriched by many fibroblasts.*

variables. Between control and treatment group patients, no drop out during the study was recorded.

Urinary incontinence was detected in 80% of the patients (n=16) with stress component as a predominant symptom. Dyspareunia improvement was greater in the TG than in the CG. According to the VAS, the mean value in the TG at baseline was 6±1 while at the 12-week follow-up it was 3.65±0.79; the mean value in the CG was 5.6±1.2 while at the 12-week follow-up was 5.55±0.86. The VHI score in TG improved from baseline from 12.6±1.8 to 17.7±0.64 at the 12-week follow-up; meanwhile in CG, VHI values did not show any differences from 11.9±1.1 at baseline to 11.3±0.78 at the 12-week follow-up.

A B

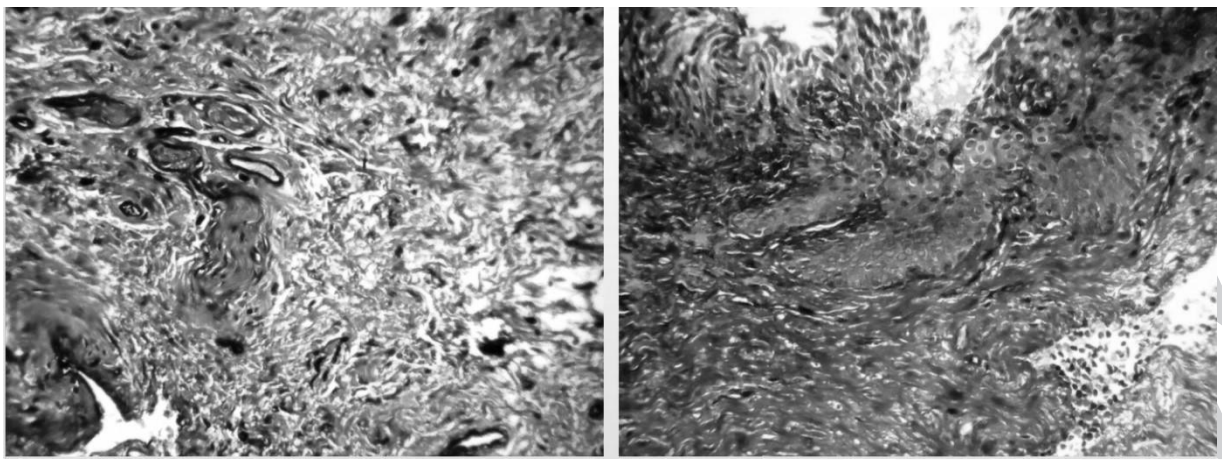

Fig. 2. *Modified Masson's trichrome stain vaginal sample. Pre-treatment (**A**) and 3 months after the injection of NCLHA + CaHa (**B**). Before the treatment the stroma appeared relatively dense with disorganized collagen, while 3 months after the injection protocol with NCLHA plus CaHA the epithelium appeared constituted by several layers of cells, and in the lamina propria an increase of collagen was clearly observable.*

ICIQ UI SF, used to evaluate urinary incontinence symptoms, showed a significant improvement in the TG at the end of the treatment protocol, the mean value was 14±2.2 at baseline and to 9.5±1.7 at the 12-week follow-up; the ICIQ UI SF did not improve in the CG, the mean value was 13.5±2.2 at baseline and 13.6±1.9 at the 12-week follow-up.

Sexual function, evaluated using FSFI score, showed an important improvement in the TG in all the points assessed: desire, arousal, lubrication, orgasm, satisfaction and pain, meanwhile the FSFI score did not show relevant differences in the CG. The results are shown in Table II.

Clinical evaluation according to the pre and post-operative pictures showed a significant improvement in the quality and tropism of the vaginal mucosa in all the TG. The histological evaluation of the samples from the patients in the TG demonstrated a significant improvement in the appearance of the epithelium. The results are shown in Figs. 1 and 2. Data analysis demonstrated an important clinical and histological improvement from baseline in all points assed in TG, while the patients in CG did not have any relevant clinical or histological improvement. No complications, side effects, local reactions or systemic complications were reported in either the TG or CG.

DISCUSSION

In the skin, the action of the CaHa in combination with or without HA scaffold is well described in literature (5, 6). Our results indicate a robust statistical significance of the improvements of symptoms, both objectively, by the use of the VHI, ICIQ UI ST, and subjectively, with a VAS of symptoms, 3 months after treatment in the treatment group. Clinical examination showed an important improvement in vaginal mucosa quality and tropism in all TG patients referring to FSFS, ICQ-SF, VAS scores and by pre- and post-operative pictures analysis. All TG patients reported an improvement in the sexual function and have also shown an improvement in urinary incontinence symptoms. A comprehensive analysis of VAS and VHI data showed how treatment satisfaction was greater in the TG compared to the CG. Moreover, important histological changes were demonstrated by the blinded pathologist in all the samples in the TG. Histological and histochemical

studies showed a significant recovery in the vaginal tissue (epithelium and stroma), and these changes appear to be related to the bio-simulative effect of NCLHA plus CaHA.

The injection of NCLHA plus CaHA in the anterior third of the vagina wall showed the ability to activate collagen and elastin production at a molecular level, restoring all the vaginal functions, such as secretion, absorption, elasticity and lubrication, as well as improving the vaginal epithelium thickness. Furthermore, once the thickness of the vaginal epithelium is re-established at the level of the anterior vaginal wall, the coaptative mechanism of the urethra is restored, and this causes a subjective and objective improvement in urinary incontinence. Moreover, also the application at the level of vulva allows to recover its shape and function which is altered in the GSM.

Treatment with NCLHA plus CaHA is affordable, safe, well tolerated and can offer patients important improvements in vaginal tropism and intrinsic and extrinsic continence mechanisms. Longer follow-up and a larger co-hort should be included in further well-designed studies in order to corroborate our findings.

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