

Elastosonographic Changes in Patients with Peyronie's Disease, before and after Treatment with a Compound Based on *Ecklonia bicyclis*, *Tribulus terrestris*, and Water-Soluble Chitosan

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Abstract

Several diagnostic techniques have been proposed during the time for the diagnostic evaluation of Induratio Penis Plastica (IPP), due to poor sensitivity and specificity of standard B-Mode Ultrasonography. Shear-Wave elastosonography (SWE) is a relative new ultrasound technique, already used for several organs, which allows evaluating the rigidity of the tissues. Furthermore, we used a compound consisting of *Ecklonia bicyclis*, *Tribulus terrestris*, and Biovis (water-soluble Chitosan) with antifibrotic and antioxidant properties and analyzed the elastosonographic variations following the assumption of the compound. From March 2016 to April 2017, 40 patients were recruited with a mean age of 62. All patients underwent elastosonographic measurements at the start of treatment. Subsequently each patient took 1 tablet of the compound of *Ecklonia bicyclis*, *Tribulus terrestris*, and Biovis (water-soluble Chitosan) for 6 months. All patients underwent elastosonographic investigation at the end of treatment. The elastosonographic measurement prior treatment, showed that the average of the three measurements (proximal, medial and distal), was $37.05 \text{ KPa} \pm 7.76 \text{ SD}$ and $38.8 \text{ KPa} \pm 10.11 \text{ SD}$ for the left and right cavernous body, respectively. The examination at the end of the six months of treatment, with the compound showed that the average values of fibrosity were $31.07 \text{ KPa} \pm 7.5 \text{ SD}$ and $30.86 \text{ KPa} \pm 11.4 \text{ SD}$ for the left and right cavernous body respectively, demonstrating a decrease in fibrosity that was statistically significant ($P < 0.01$). Our data demonstrate that taking the compound for six months reduces the stiffness of the corpora cavernosa.

Keywords

Peyronie's Disease, Elastasonography, *Ecklonia bicyclis*, Soluble Chitosan, *Tribulus terrestris*

1. Introduction

“Peyronie's Disease” (PD) is a connective tissue disorder that involves the tunica albuginea, the thick sheath of tissue surrounding the cavernous bodies (corpora cavernosa) of the penis, and is characterized by the formation of an inflammatory plaque that becomes increasingly fibrous and inelastic with time [1] [2] [3].

PD affects males around 50 years of age, with an incidence that varies between 3.2% and 13%. However, more recent studies have highlighted a greater incidence of the disease and related symptoms, suggesting a higher prevalence rate to that historically reported [4] [5]. The progression of the pathology is rather complex and irregular, characterized by phases of development, of spontaneous remission and of stabilization, which makes the evaluation of the therapeutic efficacy of a given compound all more complicated [6].

The microstructural alterations at the level of the tunica albuginea of the penis heavily reflect the patient's symptomatology, which varies from: curvature and/or deformation of the penis (94%) (sometimes making penetration impossible), penile pain during erection (20% - 70%), and erectile dysfunction (35%), all of which are sometimes associated with a depressive state of the individuals found in 48% of cases [7] [8].

Surely the diagnostic and therapeutic phases represent the most critical phases of PD.

To date, B-mode ultrasound, despite having several limits, is still the diagnostic test most widely used [9] [10]. The main ecographic limits consist in not being able to recognize isoecogene plaques compared to the surrounding parenchyma, and in not being able to highlight fibrotic zones but only calcified plaques, which, as a result, give numerous false negatives [11] [12] [13].

Another aspect, under heated debate, is certainly related to the therapeutic treatment. In fact, although several therapeutic protocols have been established over the years, which began from the one introduced by Francesco Gigot de La Peyronie, with mercury and mineral water, up to the more modern protocols with interplate injection of pharmacologically active components (such as steroids, verapamil, clostridium collagen, interferon) [14] [15] associated or not to topical treatments, such as Extracorporeal Shockwave Therapy (ESWT) and Vacuum Device, none of these has individually been more effective than others in contrasting the disorder [16] [17].

The purpose of our study, thus, originates from these observations.

Our aim, in fact, was to demonstrate the diagnostic efficacy of “Shear Wave Elastasonography” (SWE) in highlighting areas of fibrosis, at a premature stage,

i.e. before the formation of an organized plate, and therefore in “correcting” the false negatives, which would result by a B-mode evaluation [18] [19]. Furthermore, we were also able to demonstrate the therapeutic efficacy of SWE, both in terms of decrease in symptomatology and in terms of decrease in fibrosis, by exploiting the widely demonstrated natural properties of a compound based on *Ecklonia bicyclis*, *Tribulus terrestris*, and Biovis (water-soluble Chitosan) [20].

The ultimate goals of our working group were thus to:

- obtain an early and objective diagnosis of the pathology by investigating on the degree of fibrosity of the cavernous bodies thanks to the novel and promising technology of shear wave elastosonography, and
- adopt a medical and non-invasive therapy to arrest the progression of PD.

2. Materials and Methods

The clinical survey was conducted from March 2016 to April 2017.

Sixty-five patients were initially enrolled in the study. All patients had been diagnosed with Peyronie’s Disease 12 months before the beginning of the study. Patients underwent self-photography and an objective examination.

Inclusion and exclusion criteria were established in order for patients to be enrolled in the survey (Table 1).

According to the criteria described above, 25 patients were excluded (10 DM; 5 with other treatments for PD; 7 for penile fractures/traumas; 3 for pelvic-retroperitoneal RT) while 40 patients, with a mean age of 62 years old, were enrolled as the final data set. All participants signed an informed consent and the study was conducted according to the Helsinki declaration. Once enrolled in the study, all participants were submitted to the IIEF15 standardized questionnaire. The principal components analysis identified five factors: erectile function, orgasmic function, sexual desire, intercourse satisfaction, and overall satisfaction, which assessed the degree of erectile dysfunction (ED) [21].

Table 1. Inclusion and exclusion criteria. Acronyms: DM: Diabetes Mellitus. ED: Erectile Dysfunction; PD: Peyronie’s Disease.

Inclusion criteria	Exclusion criteria
Age > 18 years	DM
Clinically stable pathology for at least 12 months before the survey	Metabolic disorders
Presence or absence of concomitant ED	Smoke
	Alcoholism
	Penile fractures or traumas
	Pelvic-retroperitoneal radiotherapy
	Other medical or surgical treatments for PD
	Pelvic-penile surgery

Subsequently underwent an ultrasound scan by using the SWE technique. All measurements were performed with the GE Logic S8 ultrasound system with a linear probe and frequency of 10 MHz. The results of the measurements were expressed in KPa (kilo-Pascal), by reporting a quantitative value of the stiffness of the cavernous tissue; at the same time, we were also able to observe the qualitative stiffness of the tissue by way of a color map of the tissue examined (Figure 1).

Measurements were performed by a single tester and all patients adopted a supine position at the time of the examination. The ultrasound examination was performed both in 2D mode and SWE mode. All images were acquired from the dorsal region of the penis of each individual cavernous body, separately, with the penis in flaccid conditions.

All scans were performed using the longitudinal axis. The cavernous body was divided into three portions: proximal, medial and distal. Three different Regions of Interest (ROI) were calculated for each portion. The ROI consists of a circular section whose limits correspond to the upper and lower part of the tunica albuginea.

All patients were administered the tradamixina (trademark symbol in apix) compound (150 mg of the seaweed *Ecklonia bicyclis*, 396 mg of *Tribulus terrestris*, and 144 mg of D-glucosamine and N-acetyl-D-glucosamine) for a period of 6 months, with the dose of 1 tablet/daily.

3. Statistics

Statistical analysis was performed using the SPSS processing software version 20.0.

The analysis of continuous variables was evaluated by the paired t-test for unpaired data.

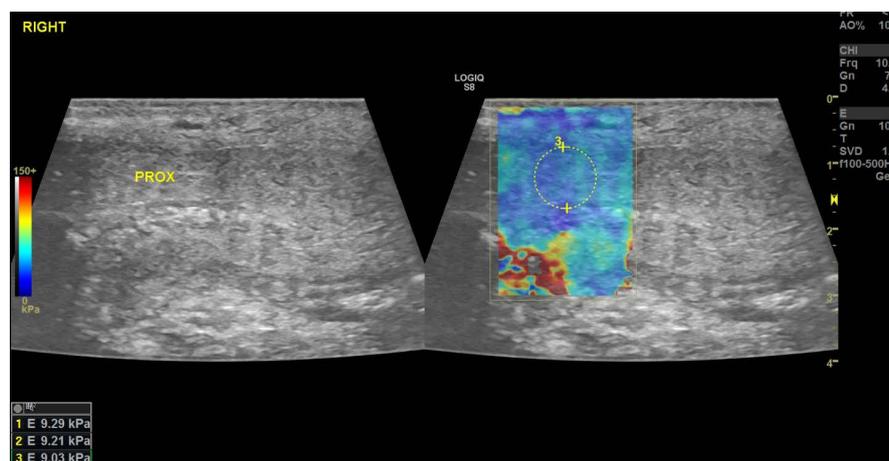


Figure 1. Representation of the ultrasound scan of the right cavernous body (proximal portion), by performing three measurements, as per protocol. The results of the measurements, expressed in KPa, are shown in the lower left corner. The chromatic mapping gives us a qualitative indication of the “stiffness” of the cavernous body.

Spearman's rank correlation coefficient was also used to test the correlation between the quantitative value expressed in KPa of the SWE and the IIEF-15 value, before and after treatment.

Data were reported as mean \pm standard deviation (SD) and normal p values were presented.

For all statistical analyses the level of significance was set at $p < 0.01$.

4. Results

All 40 patients enrolled in the study managed to complete the scheme correctly. The mean age of the patients was 62 years old (SD \pm 12.21).

The analysis of the results obtained from the submission of the IIEF-15 questionnaire, prior treatment, and adopted to evaluate a possible associated ED status, showed that in 65% of patients (N = 26) there was a slight ED, in 20% of patients (N = 8) a moderate ED and in 15% of patients (N = 6) no type of ED at all (refer to **Table 2** below for major details).

The quantitative analysis (expressed in KPa), which was performed by shear wave elastosonography (SWE), prior treatment, showed that the average of the three measurements (proximal, medial and distal), was 37.05 KPa \pm 7.76 SD and 38.8 KPa \pm 10.11 SD for the left and right cavernous body, respectively (**Figure 2**).

Table 2. Results of the IIEF-15 questionnaire compiled by patients, prior treatment.

IIEF-15 value	Number of patients
26 - 30 absence of ED	6 patients
17 - 25 slight ED	26 patients
11 - 16 moderate ED	8 patients
<10 severe ED	No patient

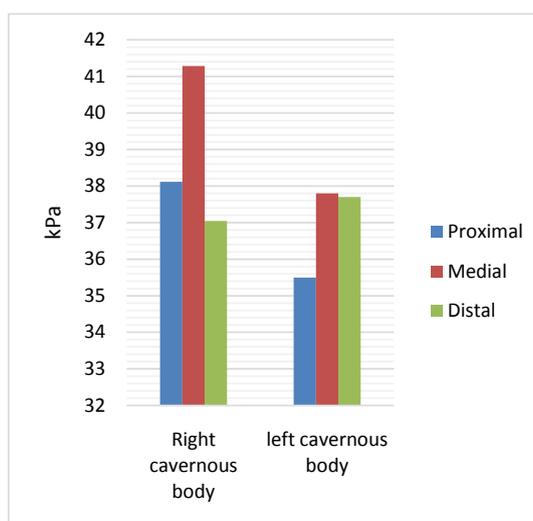


Figure 2. Evaluation of the average measurements of the proximal, medial and distal region of each cavernous body PRIOR TREATMENT.

By the end of the therapeutic treatment, each patient correctly completed the IIEF-15 questionnaire for the evaluation of sexual function and was re-exposed to an ultrasound examination with the shear wave elastosonography technique.

The quantitative analysis, evaluated by SWE ultrasound examination at the end of the six months of treatment, with the compound based on *Ecklonia bicyclis*, *Tribulus terrestris* and water-soluble Chitosan, showed that the average values of fibrosity were 31.07 KPa \pm 7.5 SD and 30.86 KPa \pm 11.4 SD for the left and right cavernous body (Figure 3), respectively, demonstrating a decrease in fibrosity that was statistically significant ($P < 0.01$; refer to Figure 4 and Figure 5 for more details).

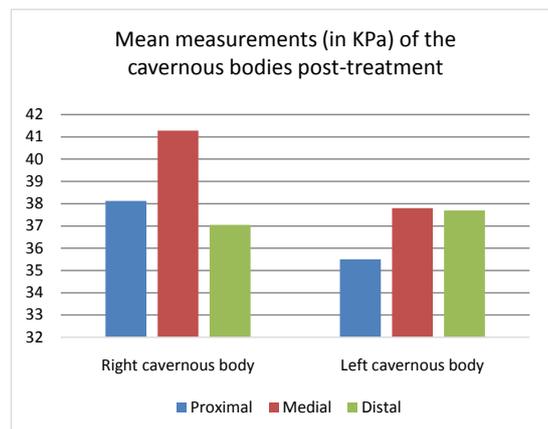


Figure 3. Evaluation of the average measurements of the proximal, medial and distal region of each cavernous body post-treatment.

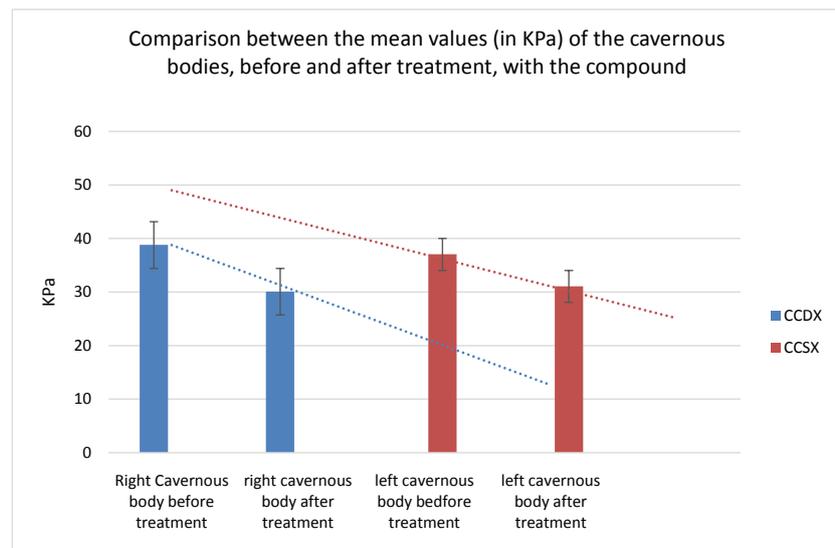


Figure 4. Comparison of the mean values expressed in KPa of the cavernous bodies (left: orange bars; right: blue bars), before and after treatment with the compound based on *Ecklonia bicyclis*, *Tribulus terrestris*, and glucosamine oligosaccharide (tradamixina compound), which shows a reduction in KPa values (expression of the degree of fibrosis of the cavernous body) that is statistically significant.

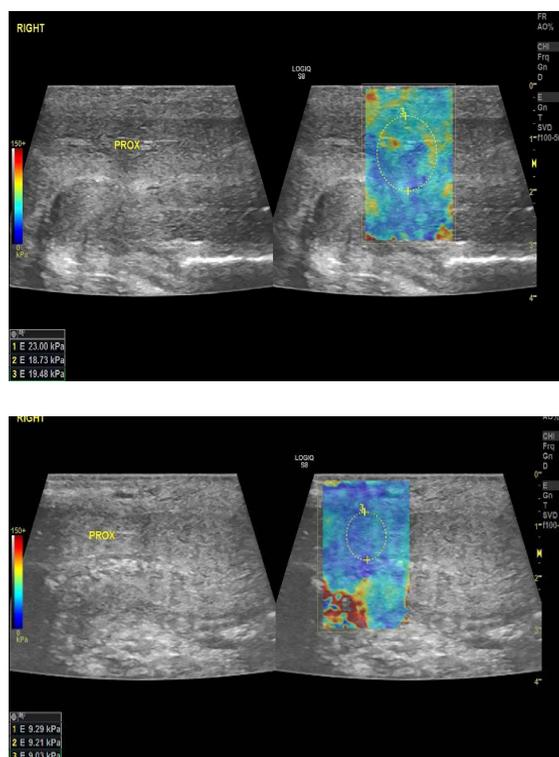


Figure 5. Comparison of two elastosonographic examinations performed, before and after treatment, showing a statistically significant decrease in the “fibrosity” values expressed in KPa.

We obtained an average score of 18.7 ± 3.2 , following the compilation of the IIEF-15 questionnaire at the end of the 6 months of treatment.

As shown in **Figure 6**, we found a statistically significant improvement ($P < 0.01$) in mean values of the IIEF-15 score by the end of the six months of treatment with the compound under examination.

5. Discussion

Despite many non-surgical therapeutic treatments available to date, none of these has shown to be capable of acting both on the pathophysiological mechanism and on the regression of the disease, but only to resolve the acute and inflammatory phase characterizing the evolutionary path of PD. Surgical therapy, on the other hand, seems to be the only definitive solution in an advanced state of the disorder.

Our working group, thus, decided to use the compound consisting of *Ecklonia bicyclis*, *Tribulus terrestris* and Biovis (water-soluble Chitosan), and to exploit the potential of these natriuretic principles, which, we postulated, could stop the aforementioned pathophysiological cycle.

In particular, *Ecklonia bicyclis* is a brown edible seaweed (Phaeophyceae, Lessoniaceae family), which is part of the phlorotannin family and has shown to have remarkable antioxidant properties. It is rich in minerals and essential

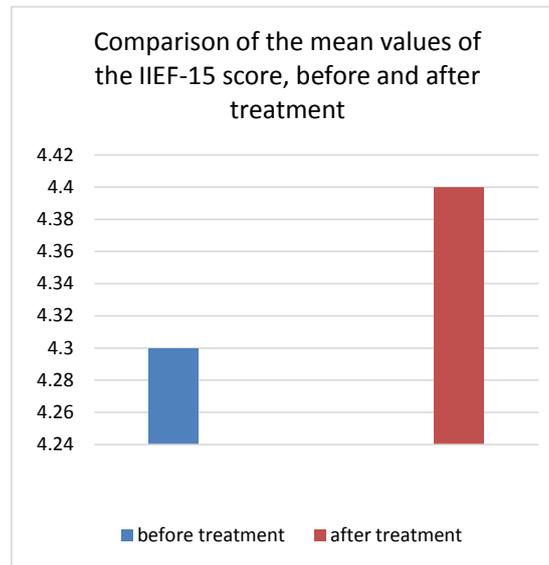


Figure 6. Comparison of the mean values of the IIEF-15 score, before and after treatment with the compound. Data are expressed as mean \pm SD. The mean values of IIEF-15 were 20.07 ± 3.74 and 23.6 ± 2.9 before treatment and after six months of therapy, respectively.

elements, but above all contains a specific class of polyphenols called “phlorotannins”. The importance of polyphenols (produced by the secondary metabolism of algae) is expressed by their antioxidant function, which protects cells and tissues from the harmful effects caused by ROS (reactive oxygen species).

More specifically, three components (Dieckol, Phlorofucoeckol and Bieckol), contained within the seaweed *Ecklonia bicyclis* and made up of eight or more phenolic rings, act as scavengers of free radicals, arresting the oxidation process that is activated in the pathology in early-onset. The scavenger activity of free radicals by these phlorotannins has been shown to be 10 - 100 times more powerful than in any other polyphenol found in terrestrial plants. In addition, phlorotannins, unlike other common polyphenols that have a rather short half-life (about 12 hours), thanks to their 40% liposolubility are both able to cross the blood-brain barrier and to perform a much longer-lasting action [20] [22].

The compound we decided to use also presents other important properties, making it very useful even when there is a drop in libido (which inevitably follows a variable degree of erectile dysfunction). Our study showed an improvement in the score of the IIEF 15 questionnaire, which underlines an improvement in both erection and sexual desire thanks to the properties of *Tribulus terrestris* and Biovis. The first, thanks to the hormone-like properties (it acts as a substitute for testosterone), increases libido; the second, which contains polymers of water-soluble Chitosan, acts as a stimulant and inducer of a mediator, nitric oxide (NO), favoring the natural release of NO and obtaining a greater concentration of this mediator in the cavernous bodies.

More in detail:

Tribulus terrestris is a plant belonging to the Zygophyllaceae family. The most important properties of this plant are related to its stimulating action on the production of androgen hormones, which are very important for the regulation of libido, development of muscle mass and sexual characteristics. These effects are attributed to a group of organic substances with hormone-like activity called saponins. The seeds of *Tribulus terrestris* are indeed rich in protodioscin, a steroidal saponin that acts by increasing the endogenous production of testosterone, dihydrotestosterone, luteinizing hormone (LH), dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEA-S), resulting in an increased spermatogenesis and consequent increase of the libido in both experimental animals and humans [23] [24].

The Biovis polymer of D-glucosamine and N-acetyl-D-glucosamine, on the other hand, has been shown to be a valid inducer of nitric oxide synthase (NOS), acting on both the non-adrenergic-non-cholinergic (NANC) cells of the nervous system and on the cells of the endothelial system with the consequent notable increase of NO [25].

As results from the literature, shear wave elastosonography (SWE) has already been previously applied in the treatment of liver, thyroid, breast and musculoskeletal diseases [26].

Here we adopted this novel technique to measure the tissue stiffness of the cavernous bodies of the penis *in vivo*, which is unprecedented, to the best of our knowledge.

SWE can be considered an extension of the normal method adopted by the physician in evaluating the elastic tension and stiffness of the penis obtained by a morphological examination. During SWE, the US-probe is applied with a constant pressure on the target area, the tissue behind the target area being somewhat deformed; it goes without saying that different tissues have a different degree of deformation. By means of a specific software, the tissue deformation is expressed on the US monitor as a color variation and presents a specific value expressed in Pascal (International System derived unit of pressure). In particular, we based our study on the assumption that the cavernous bodies of the penis, affected by Peyronie's disease, tend to be stiffer than normal [26] [27].

- The results obtained in our study are promising since we were able to demonstrate that a 6-month therapy with a natriuretic complex, based on *Ecklonia bicyclis*, *Tribulus terrestris* and Biovis (water-soluble Chitosan), was sufficient to decrease the fibrosity of the cavernous bodies of the penis in a statistically significant way. However, our results are preliminary at this stage and more should be done to overcome the limits of our study, which are manifold: the sample size of the present study was small and should thus be extended;
- The follow-up over time was only 6 months; a longer follow-up should be conducted to confirm our data, and a standardization of the penile shear wave elastosonography method should be promoted.

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