

## High Concentration of Reticular Fibers in Periurethral Region of the Prostatic Urethra from Patients with Benign Prostatic Hyperplasia as Revealed by Stereology

Alta Concentración de Fibras Reticulares en la Región Periuretral de la Uretra Prostática de Pacientes con Hiperplasia Prostática Benigna Revelada por Estereología

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**SUMMARY:** The goal of this investigation was to analyze and quantify changes of the reticular fibers in the prostatic urethra of patients with benign prostatic hyperplasia (BPH) and compare with a control group. Prostatic urethra tissue samples were obtained from ten patients (age range 65 to 79 years, mean 66) with clinical symptoms of bladder outlet obstruction who had undergone open prostatectomy. The ten control group samples (urethral tissue samples from the transitional zone) were collected from prostates obtained during autopsy of accidental death adults of less than 25 years. The Vv of the reticular fibers was determined with stereologic methods from 25 random fields per sample using the point-count method with a M-42 grid test system. The quantitative data were analyzed using the Kolmogorov-Smirnov and Mann-Whitney U tests. The Vv (mean±SD) in the control and BPH groups respectively were: 23.4± 1.8 and 30.3 ± 1.2 (0.001). BPH cause significant increase of reticular fibers in prostatic urethra.

**KEY WORDS:** Prostatic urethra; Reticular fibers; Stereology.

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### INTRODUCTION

The benign prostatic hyperplasia (BPH) is urological disorder, most common in men over 50 years of age (Berry *et al.*, 1984; Chagas *et al.*, 2002), is associated with complex, not well understood, interactions between acinar epithelial cells and their supportive stroma (Costa *et al.*, 2004; Babinski *et al.*, 2003; 2007). Epithelial–stromal interactions and stromal extra cellular matrix (ECM) components also play key roles in normal prostate physiology and in tumor growth (nodules) (Cunha, 1994; Nagle, 2004).

The majority of the TZ prostatic urethral injuries have a compressive origin from the BPH mass (McNeal, 1978; Roehrborn & McConnell, 2002; Chagas *et al.*; Costa *et al.*), thus, the nodular growth occurs in the TZ in >70% of the cases and compresses the prostatic urethra resulting in bladder outlet obstruction (BOO) (McNeal, 1978, 1990, 1997). It is well known that BOO in BPH results from mechanical obstruction by the enlarged prostate and the tone

of the prostate smooth muscles in the prostatic tissue (Caine, 1986; Ichihyanagi *et al.*, 1999). As a consequence of these changes, lower urinary tract symptoms (LUTS) in men usually occur and are considered a consequence of BOO due to BPH (Ichihyanagi *et al.*).

The extracellular matrix of the prostatic urethra has received less attention than smooth muscle disposition and innervation (Dorschner *et al.*, 2001; Yucel & Baskin, 2004; Karam *et al.*, 2005). So, the structure of this region has still not been properly studied in the normal prostatic urethra, nor compared with hyperplastic samples. A better knowledge of the histological components in the normal and the hyperplastic prostatic urethra is necessary. Thus we studied the volumetric density (Vv) of the fibroelastic element (reticular fibers) in the prostatic urethral samples from patients with BPH mass and control (normal) groups, to define the role of the extracellular matrix components in LUTS.

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## MATERIAL AND METHOD

**Ethics procedures.** This study complies with the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh, 2000). Our Internal Review Board approved study guidelines. Also, the protocol received approval by the Ethics Committee on Human Research of the State University of Rio de Janeiro. All patients provided written informed consent for the use of prostatic tissue material for research prior to inclusion in the study.

**Groups and samples.** BPH tissue samples of the transitional zone of the urethra were obtained from ten patients without symptoms of BOO who did not undergo any treatment for symptomatic BPH. Each patient had undergone open prostatectomy (retropubic or transvesicular). All patients studied had prostates larger than 40g (mean 60), a flow rate of 12 mL/s and an International Prostate Symptom Score (IPSS) of 12 or greater. Patient age range was 63 to 79 years (mean 66) and all patients had shown histological diagnosis of BPH with no foci of prostatic carcinoma.

The control samples were obtained from autopsies of young males ranging in age from 18 to 30 years (mean 24), who died of unrelated causes to the urogenital system. Samples were taken from the transitional zone of the urethra of ten prostates. A sagittal section from the anterior region to the lumen of the prostatic urethra was taken exposing the verumontanum. This anatomic landmark was used to excise urethral fragments (1X1cm) accurately from the transition zone (McNeal, 1978; 1990). The time elapsed between death and fixation of the excised controls was less than six hours.

**Histological procedures and morphometrical method.** The samples of prostatic urethra tissue were immersed in 10% formalin solution for 24 hours and then embedded in paraffin. All samples were initially stained with hematoxylin-eosin and examined by a pathologist to detect any foci of carcinoma and to exclude samples with artifacts. A pathologist from the University Hospital, who was not involved in the research protocol, analyzed the prostate samples obtained in the surgical unit. Then, a pathologist from the Urogenital Research Unit confirmed the diagnosis.

From each prostate, five different samples of the prostatic urethra were taken from the transitional zone. From each sample, five different sections were selected. Five random fields were evaluated from each section, resulting in the analysis of 25 test areas from each prostatic urethra, totaling 250 fields that were analyzed for reticular fibers in each group. For the stereologic analysis, the 5µm sections were stained with the Gomori's reticulin.

The analyzed fields were then digitized to a final magnification of X400 using a video camera coupled to a light microscope (BH-2 Olympus). The selected histological areas were then quantified by applying a test-grid system (M42) on the digitized fields on the screen of a color monitor (Sony). From stereologic principles in isotropic tissue, the area distribution of a given structure, as determined on a two-dimensional section of the structure, is proportional to the volume distribution of the structure. The volume density of the histological components was calculated as  $V_v = P_p/P_t$ , where  $V_v$  was the volume density,  $p$  was the tissue component under consideration,  $P_p$  was the number of test points associated with  $p$ , and  $P_t$  was the number of points in the test system. The stereologic methods have been described in detail previously (Weibel *et al.*, 1966; Gundersen *et al.*, 1988).

**Statistical analysis.** The data were analyzed using the Kolmogorov-Smirnov test to verify normal distribution (Gaussian) and variance of data, as well as, the unpaired t test to demonstrate whether the  $V_v$  differences were statistically significant.  $P \leq 0.05$  was considered statistically significant.

## RESULTS

The reported findings are exclusively of the transitional zone of the assessed human prostatic urethras. In control group, beneath the mucosa, the sub mucosa was observed containing reticular fibers interspersed with smooth muscle (Fig.1). In the BPH group, reticular fibers presented disorders in the prostatic urethra (Fig.2) with statistically, significant increase no  $V_v$  of this fibers. The relative values (% mean±SD) of the quantitative evaluation in both groups are shown in Table I.

Table I. Results of reticular fibers, quantitative evaluation (volumetric density) in prostatic urethra transitional zone.

Element	Controls	BPH Urethra	N	P Value
Reticular Fiber	23.4± 1.8	30.3± 1.2	10	0.001 *

Data presented as the mean percentage ± SD.  
\* Statistically significant, Mann-Whitney U test.

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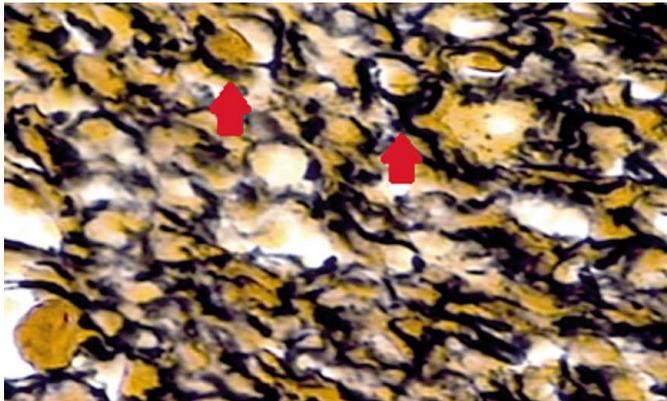


Fig. 1. Photomicrograph of a transition zone prostatic urethra section from control group illustrating the reticular fibers arrangement (red arrows) in sub mucosa layer. Note their variable thickness. Gomori's reticulin staining, original magnification X40.

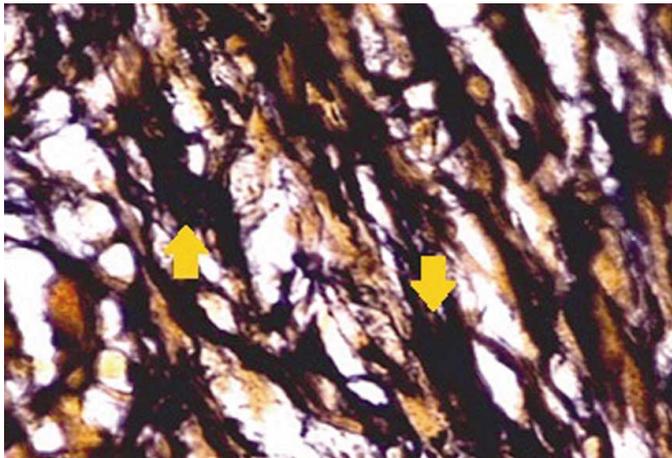


Fig. 2. Photomicrograph of prostatic urethra from patient in BPH group showing damage stroma with increase of reticular fibers (yellow arrows). Note in some areas, the fibers are disorganized. Gomori's reticulin staining, original magnification X40.

## DISCUSSION

The prostatic urethra is lined by transitional epithelium (Tanagho & McAninch, 2000). Regional differences may exist and vary from squamous, stratified or pseudostratified columnar epithelium (Orlandini & Orlandini, 1989). These variations may extend into the prostatic ducts (Roehrborn & McConnell). It is primarily composed of a mucosal layer covered by urothelium. Beneath the basal membrane a layer of connective tissue (submucosa) (Tanagho & McAninch) contains vascular sinusoids, cells (mainly fibroblasts), an extracellular matrix of collagen, proteoglycans, reticular and elastic fibers, glycoproteins and an inner longitudinal and an outer circular layer of smooth muscle (Tanagho & McAninch; Bastos *et al.*, 2004; Dorschner *et al.*; Yucel & Baskin).

The prostatic transitional zone (TZ) is particularly relevant when considering prostate pathology. Thus, there are several conflicting opinions concerning the relationship between the size of the hyperplastic prostate and the grade of urethral compression (Shapiro *et al.*, 1992; Kaplan *et al.*, 1995; Terris *et al.*, 1998; Ichiyangi *et al.*). The relationship between the International Prostate Symptom Score (IPSS) and the prostate total volume suggests that the bigger prostate impinges more making the symptoms more severe (Kojima *et al.*, 1997). On the other hand, the lack of relationship between the total prostatic volume and BOO suggests that the symptoms and urodynamic outcomes of the infra-vesical obstruction from HPB is more associated to the enlargement of the prostatic transitional zone than to the total prostatic volume (Kaplan *et al.*; Terris *et al.*; Ichiyangi *et al.*). The relationship between the histological changes in the prostatic urethra and the beginning of the obstructive symptoms is not completely recognized.

The extracellular matrix is very important in the development of tissues and organs, rebuilding and repair of damages, and maintenance of the tissue normality (Kielty *et al.*, 2002; Hay, 1991; Eckes *et al.*, 2000). Therefore, the location and arrangement of the extracellular matrix can be associated with the functional features of each kind of tissue, pointing out their particular biomechanical proprieties (Kielty *et al.*; Hay; Eckes *et al.*). Changes in the elements of the extracellular matrix of an organ also change these proprieties (Peters *et al.*, 1997; da Silva *et al.*, 2002). The observed human male urethras presented a uniform gross anatomical appearance, while changes in the extracellular matrix could be noted in its various segments (membranous, spongy and glandular), justifying the functional (Hsu *et al.*, 1994; da Silva *et al.*) and pathological differences (Singh & Blandy, 1976; Baskin *et al.*, 1993; da Silva *et al.*). A significant increase of reticular fibers was found. The external pressure from the hyperplastic nodules of the transitional zone, probably, creates a urethral rebuilding. This process imposes a large urethral distension together with a characteristic increasing in length (longitudinal and antero-posterior axis) of the proximal prostatic urethra, damaging the mucosa and sub mucosa (McNeal, 1997). McNeal (1997) reported that this change could take place due to the urethral position between the two transitional zone masses. This could compress the urethra and create an enlargement in the urethral mucosa, which increases as much as the hyperplastic nodules expand. Thus, the fibrous elements of the extracellular matrix should have an important role in preservation of the normal structure of the prostatic urethra. However, this connection has not been related previously.

The increasing of the reticular fibers affects the prostatic urethra by diminishing its resistance. The rebuilding process of the extracellular matrix is likely similar for the transitional zone parenchyma, from the response in the stromal hyperplastic nodules (Chagas *et al.*; Costa *et al.*). Hence, the utmost important modifications in the prostatic urethra in patients with BOO for HPB are in the extracellular matrix. These changes are associated to the great enlargement of urethra and the increasing of the external pressure from the prostatic hyperplastic nodules of the transitional zone (McNeal, 1972).

The results of this study offer new information about urethral morphology (McNeal, 1972; da Silva *et al.*), mainly with reference to damage of the prostatic urethra by the hyperplastic nodules of the prostatic transitional zone

(McNeal, 1997; Chagas *et al.*; Costa *et al.*; Babinski *et al.*, 2007) and the urodynamic outcome concerning BOO (Ishigooka *et al.*, 1996; Gosling, 1997; Kojima *et al.*; Terris *et al.*; Ichiyonagi *et al.*; Inui *et al.*, 1999). Briefly, the increasing reticular fibers could be associated with resistance and urethral complacency.

In conclusion, BPH nodules cause compression forces in prostatic urethra and consequently induces a remodeling process of the extracellular matrix with significant increase of reticular fibers in prostatic urethra.

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**RESUMEN:** El objetivo de esta investigación fue analizar y cuantificar los cambios de las fibras reticulares en la uretra prostática de pacientes con hiperplasia prostática benigna (HPB) y compararlo con un grupo control. Muestras de tejido de uretra prostática se obtuvieron de diez pacientes (rango de edad 65 a 79 años, media 66) con síntomas clínicos de obstrucción del tracto urinario inferior que se habían sometido a prostatectomía abierta. Las diez muestras del grupo de control (muestras uretrales de tejido de la zona de transición) se obtuvieron de próstatas durante la autopsia de sujetos adultos con muerte accidental menores de 25 años. El Vv de las fibras reticulares se determinó con métodos estereológicos de 25 campos al azar por muestra utilizando el método de valor de conteo con el sistema M-42. Los datos cuantitativos se analizaron mediante la prueba de Kolmogorov-Smirnov y Mann-Whitney U. El Vv (media  $\pm$  DE) en el grupo control y BPH, respectivamente, fueron:  $23,4 \pm 1,8$  y  $30,3 \pm 1,2$  (0,001). La BPH causó aumento significativo de fibras reticulares en uretra prostática.

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**PALABRAS CLAVE:** Uretra prostática; Fibras reticulares; Estereología.

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