ELSEVIER

VOLUMETRIC DENSITY OF ELASTIC AND RETICULAR FIBERS IN TRANSITION ZONE OF CONTROLS AND PATIENTS WITH BENIGN PROSTATIC HYPERPLASIA

WALDEMAR S. COSTA, ARLINDO M. DE CARVALHO, MARCIO A. BABINSKI, MAURÍCIO A. CHAGAS, AND FRANCISCO J. B. SAMPAIO

ABSTRACT

Objectives. To analyze the volumetric density (Vv) of elastic and reticular fibers in the transition zone of controls and patients with benign prostatic hyperplasia (BPH).

Methods. Prostatic tissue samples were obtained from 25 patients (age range 63 to 79 years, mean 68) with clinical symptoms of bladder outlet obstruction who had undergone open prostatectomy. The control group was composed of 25 transition zone samples from prostates obtained during autopsy of adults aged younger than 30 years (killed in accidents). The Vv of the elastic and reticular fibers was determined in 25 random fields per prostate, using the point-count method with an M-42 grid test system. The data were analyzed using the Kolmogorov-Smirnov, Shapiro-Wilk W, and Mann-Whitney *U* tests. The Vv of these components was determined by stereologic methods.

Results. The Vv in the control and BPH groups was 12.47% ± 3.6% and 16.55% ± 9.11% in the elastic system fibers (difference not statistically significant) and 22.75% ± 1.66% and 29.33% ± 1.08% in the reticular fibers (difference statistically significant), respectively.

Conclusions. Reticular fibers showed a statistically significant increase in the BPH samples, and this finding suggests that stromal network fibers play a significant role in BPH. UROLOGY **64:** 693–697, 2004. © 2004 Elsevier Inc.

Benign prostatic hyperplasia (BPH) represents the non-neoplastic growth of the prostate that affects the male population, especially after the fifth decade of age. It has a clear relationship to age[1] and can affect 90% of men older than 90 years of age.[2] The result of this growth is a change in the prostatic histologic architecture.[3–5] These changes result in the formation of nodules that, in more than 70% of cases, are located in the transition zone.[3] Although described as hyperplasia of the prostatic glandular and stromal compartments,[6] its pathogenesis has not been entirely clarified.

It is known that the infravesical obstruction in BPH has a static/mechanical component, represented by the anatomic presence of adenoma, and a dynamic mechanism, represented by the tonus of the prostatic smooth muscles.[7] The severity of symptoms produced by BPH and the degree of infravesical obstruction, or even the International Prostate Symptom Score, do not show a relationship with prostate size,[8,9] and dynamic findings are independent of the volumetric density of the different prostatic components.[10,11] Thus, a better knowledge of the histologic components of the normal and hyperplastic prostate is required, especially regarding the extracellular matrix and its pathophysiology.

The prevalence of histologic BPH predates the incidence of clinical or symptomatic BPH.[9] Quantitative studies have described a statistically significant increase in the stromal component and important changes in the proportions of their elements during the development of BPH.[12,13] The

This study was supported by grants from the National Council of Scientific and Technological Development (CNPq-Brazil) and the Foundation for Research Support of Rio de Janeiro (FAPERJ).

From the Urogenital Research Unit, Biomedical Center, State University of Rio de Janeiro; and Department of Morphology, Biomedical Institute, Fluminense Federal University, Rio de Janeiro, Brazil

Reprint requests: Francisco J. B. Sampaio, M.D., Urogenital Research Unit, Biomedical Center, State University of Rio de Janeiro, Avenue 28 de Setembro, 87-Fundos-FCM-Térreo, Rio de Janeiro, RJ 20551-030, Brazil

Submitted: March 1, 2004, accepted (with revisions): May 14, 2004

0090-4295/04/$30.00
doi:10.1016/j.urology.2004.05.017

prostatic stroma is primarily composed of smooth muscle cells and connective tissue containing fibroblasts, collagen, and elastic system fibers, in addition to nerves, lymphatics, and blood vessels.[12,14] Studies involving animal experimentation and in vitro cell cultures have revealed that BPH is a stromal disease.[15]

The goal of the present study was to evaluate the volumetric density (Vv) of the elastic system and reticular fibers of the extracellular matrix in the prostatic transitional zone of prostate samples from controls and patients with BPH.

MATERIAL AND METHODS

The local ethics committee on human research approved the study, and all patients provided written informed consent.

BPH tissue samples of the transitional zone were obtained from 25 patients without symptoms of bladder outlet obstruction, who did not undergo any treatment for symptomatic BPH and who had undergone open prostatectomy (retropubic or transvesical). All patients studied had prostates larger than 40 g (mean 60), a flow rate of 12 mL/s or less, and an International Prostate Symptom Score of 12 or greater. Patient age range was 63 to 79 years (mean 68), and all presented with a histologic diagnosis of BPH, with no focus of prostate carcinoma.

The control samples comprised the transition zone of 25 prostates obtained from autopsies of young individuals ranging in age from 18 to 30 years (mean 24), who had died of causes not related to the urogenital system. The mean prostate weight in the controls was 25 g. A sagittal section from the anterior region to the lumen of the prostatic urethra exposed the verumontanum, and this anatomic landmark was used to excise tissue accurately from the transition zone, as described previously.[3–5] The time elapsed between death and fixation of the excised controls was less than 6 hours.

The fragments were submitted to isotropic and random orthogonal triplet probe sections for stereologic analysis.[16] Next, the samples of prostatic tissue were immersed in Bouin's fixative for 24 hours and then embedded in paraffin. Sections 5-μm thick were stained with Weigert's resorcin-fuscin with previous oxidation and Gomori's reticulin. All samples were initially stained with hematoxylin-eosin and examined by a pathologist to detect any focus of carcinoma and to exclude samples with artifacts. A pathologist from the University Hospital who was not involved in the research protocol first analyzed the prostate samples obtained in the surgical unit. Then, a pathologist from the Urogenital Research Unit confirmed the diagnosis.

From each prostate, five samples were excised from the transitional zone, and, from each sample, five different sections were selected. Five random fields were then evaluated from each section, resulting in the analysis of 25 test areas from each prostate, totaling 625 fields that were analyzed for elastic and reticular fibers in each group. For the stereologic analysis, the 5-μm sections were stained with the Weigert resorcin-fuscin technique to detect the elastic system fibers (stained violet) and Gomori's reticulin to detect the reticular fibers (stained black). The analyzed fields were then digitized to a final magnification of ×400 using a video camera coupled to a light microscope. The selected histologic areas were then quantified by applying a test-grid system on the digitized fields on the screen of a color monitor. 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 histologic components was calculated as Vv = Pp/Pt, where Vv was the volume density, p was the tissue component under consideration, Pp was the number of test points associated with p, and Pt was the number of points in the test system. The stereologic methods have been described in detail elsewhere.[17,18]

The data were analyzed with the Kolmogorov-Smirnov test and Shapiro-Wilk W test to verify normal distribution and variance of data, as well as the unpaired Mann-Whitney *U* test to demonstrate whether the Vv differences were statistically significant. $P < 0.05$ was considered statistically significant.

RESULTS

The volumetric density (Vv) of the elastic system fibers in the prostate transition zone of controls and patients with BPH was 12.47% ± 3.6% and 16.55% ± 9.11%, respectively (differences not statistically significant, $P = 0.332$; Fig. 1A,B and Table I). Because the variance was different between the two groups studied, we applied the Mann-Whitney *U* test, which did not demonstrate statistically significant differences between the mean values of the two groups. The median value for the two groups was similar. Six prostates in the BPH group (24%) presented with a Vv greater than 20%; the maximal Vv in the control group was 17.8%.

The Vv of the reticular fibers in the prostate transition zone of the controls and patients with BPH was 22.75% ± 1.66% and 29.33% ± 1.08%, respectively (differences statistically significant, $P < 0.0001$; Fig. 1C,D and Table I). The greatest concentration of reticular fibers in BPH was found in the periacinar region (Fig. 1B,D).

COMMENT

The stereologic method has been used in quantification studies[4,13,19] specifically to determine the number (or percentage) of fibrous components of the extracellular matrix.[4,18] The point counting method proved to be very efficient, avoiding the bias that frequently occurs with computerized image analyses, which may overestimate or underestimate the analyzed structures.[4,19]

The correlation between the histologic changes and the establishment of obstructive symptoms has not been clarified perfectly, because the grade of infravesical obstruction does not necessarily have a relationship to prostate size.[8] Some investigators have believed that the obstruction mechanism is mixed, with a static component formed by the anatomic presence of the adenoma and a dynamic component represented by the tonus of the prostatic smooth muscles.[7] Nevertheless, Ichiyanagi and coworkers[20] have demonstrated a decrease in the volumetric density of smooth muscles in patients with infravesical obstruction due to BPH, stressing the role of nonmuscular components in the pathophysiology of the disease.

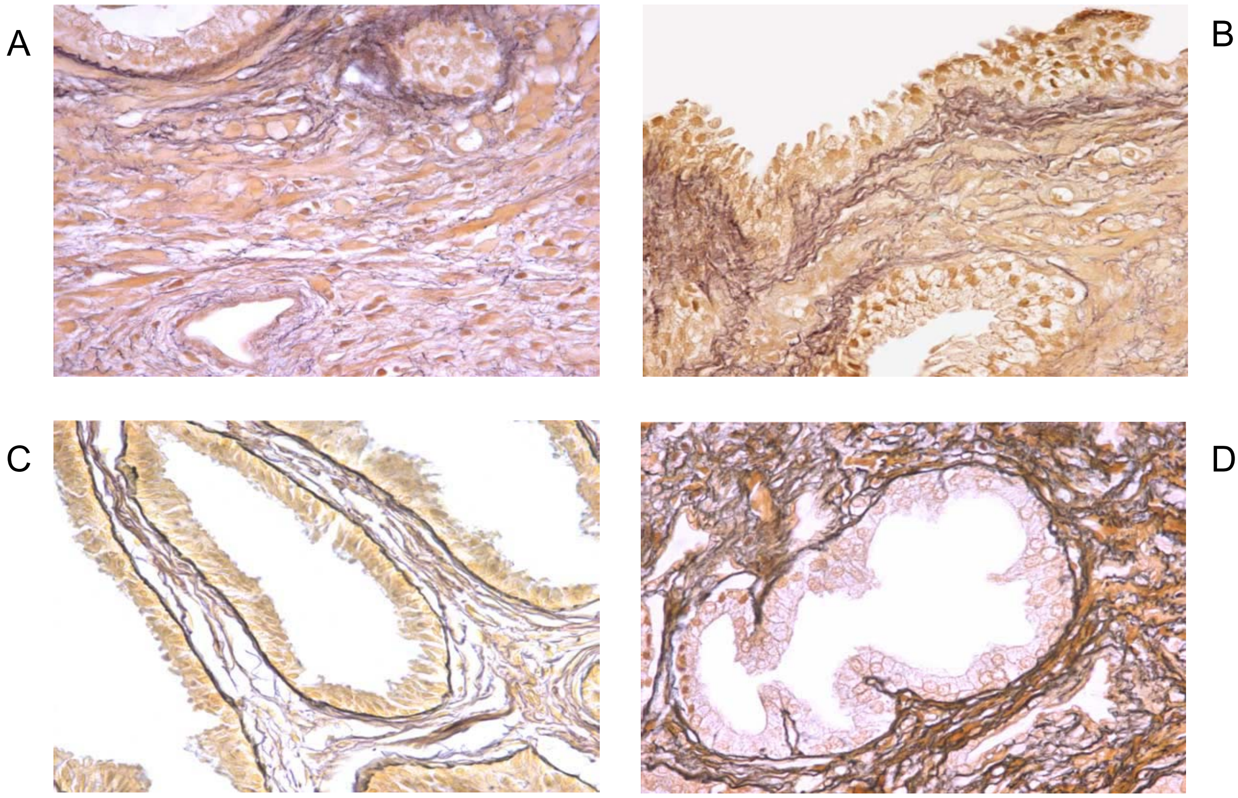

FIGURE 1. *(A) Photomicrograph of prostate from patient in control group demonstrating distribution of elastic system fibers. Weigert stain, original magnification ×40. (B) Photomicrograph of prostate from patient in BPH group demonstrating great quantity of elastic system fibers. Weigert stain, original magnification ×40. (C) Photomicrograph of prostate from patient in control group showing few reticular fibers in stroma and periacinar region. Gomori's reticulin stain, original magnification ×40. (D) Photomicrograph of prostate from patient in BPH group demonstrating important increase in reticular fibers in stroma, with condensation in periacinar region. Gomori's reticulin stain, original magnification ×40.*

TABLE I. ***Results of elastic system and reticular fiber quantitative evaluation (volumetric density) in prostatic transitional zone***

Fiber	Controls	BPH	n	*P* Value
Elastic	12.47 ± 3.6	16.55 ± 9.11	25	0.332*
Reticular	22.76 ± 1.66	29.33 ± 1.08	25	0.0001†

Key: BPH = benign prostatic hyperplasia.
Data presented as the mean percentage ± SD.
** Not statistically significant, Mann-Whitney U test.*
† Statistically significant, Mann-Whitney U test.

The fibroelastic components of the extracellular matrix are characterized by a high degree of extensibility and elastic recoil. The location and arrangement of these fibers suggest that they are functionally different, reflecting the mechanical properties of the tissues in which they occur.[21]

The correct knowledge of the quantitative changes in the extracellular matrix during the development of BPH is important for determining the best therapy in each case.[13,22] The importance of knowing the Vv of the smooth muscle cells has been previously reported[4] to understand the action of alpha-blocker drugs, which depends fundamentally on the muscle tonus mediated by $alpha_1$-adrenergic receptors[9,23] and on the density of such receptors and their properties, which differ in different prostate zones.[24] The testosterone-suppressing and dihydrotestosterone-suppressing action of antiandrogens in reducing prostate volume occurs mainly in the static component, promoted primarily by an increase in the extracellular matrix, as well as epithelial hyperplasia.[25]

The localized proliferation of the fibromuscular stroma is accepted as the first step in the development of BPH.[26] It includes significant changes in fibroblasts, capillaries,[6] collagen, elastic system fibers, and glycosaminoglycan composition.[27]

Quantitative analysis has showed a statistically significant increase in the stroma of prostates with

BPH in relation to normal prostates; however, it is not clear whether only the transition zone was analyzed. Also, the stroma was analyzed as a whole, without providing data on each of its components.[12,13] Prostatic adenoma from symptomatic men, such as in this study, would have a significantly greater proportion of stroma and lower proportion of epithelium in relation to those with asymptomatic BPH.[9,12] Studies in young individuals have showed a stroma/epithelium ratio of 2:1 in relation to BPH, in which a ratio of 5:1 was found, suggesting that BPH could actually be a stromal process.[13] In studying the prostate specimens obtained from symptomatic patients who had undergone transurethral resection of the prostate, Ishigooka *et al.*[11] demonstrated an inverse correlation between the relative proportion of muscle cells and the prostate size, as well as a positive correlation between the percentage of fibrous tissue and prostate volume, stressing the role of this component in the organ's growth. Also a weak positive relation was found between the percentage of fibrous tissue and the International Prostate Symptom Score. Because approximately 70% of the stromal component is composed of fibrous tissue elements,[11] and most research has studied the muscular element, it is necessary to provide a better clarification of the role of the elastic system and collagen.

Studies that have demonstrated a decrease in the volumetric density of smooth muscles in patients with infravesical obstruction due to BPH have stressed the role nonmuscular components play in the disease's pathophysiology.[20] It is possible that in addition to such a decrease in the smooth muscles and the inverse correlation between the proportion of muscle cells and prostate size observed in the study by Ishigooka *et al.*,[11] a constant remodeling in the elastic component also occurs so that it provides a high variance in the volumetric density of such fibers, such as was observed in the present study. Such a fact was not detected in relation to the reticular fibers.

The different kinds of collagen in the prostate extracellular matrix suggest a different role in the physiology of these fibers.[21] This hypothesis is supported by the presence of reticular fibers, which have a high degree of interaction with the ground substance, especially with heparan sulfate.

The hydrophily of these glycosaminoglycans, with a hydration (solvation) layer and the presence of sulfate radicals that interact with the collagen basic groups, binding collagen fibrils to one another, confers to these fibers different mechanical properties, increasing their resistance to compression forces.[28] This could explain the frequent increased hardness in BPH samples, with direct implication of the static component (nodule) of bladder outlet obstruction. The increase in specimen consistency found in BPH was usually observed during cleavage of the processed samples.

Carvalho and Line[29] reported that the microfibril network must also be important in providing the tissue with the compliance needed during smooth muscle contraction and elastic restoration of the resting morphology after compression forces. This not only results from the extensibility demonstrated by elastin-associated microfibrils, but also from the flexibility of its arrangement and organization.

It is known that stromal elements contribute to infravesical obstruction with a passive strength that is not regulated by the action of smooth muscular contraction.[30] Shapiro *et al.*[2] studied prostates obtained from autopsies of individuals aged 2 days to 40 years and demonstrated a progressive and statistically significant age-dependent decline in the density of smooth muscle from childhood to puberty and a statistically significant increase from puberty to 40 years of age. The reverse occurred with the connective tissue density, suggesting that up to this age, only qualitative changes were occurring, because the volumetric density of the stromal compartment remained constant. The hormonal changes involved in human development would influence the composition of the prostatic tissue elements, a fact previously demonstrated in prostatic embryogenesis.[6] However, no statistically significant alteration would occur in the quantity of elastic fibers, given the small variance observed in the control group of the present study. The high variance observed in the BPH group and the high volumetric density found in 24% of the prostates in our study suggest that the changes in the fiber network of the extracellular matrix in the hyperplastic tissue are complex and that the role of nonmuscular elements of the prostatic stroma, by increasing the passive force implied in the mechanical component of the obstruction, can make a difference when choosing the most suitable therapy.

To our knowledge, this was the first study to identify a statistically significant increase in the reticular fibers in the stroma of the transition zone in BPH prostates.

REFERENCES

1. Berry SJ, Coffey DS, Walsh PC, *et al:* The development of human benign prostatic hyperplasia with age. J Urol **132:** 474–479, 1984.

2. Shapiro E, Hartanto V, Pesman EJ, *et al:* Morphometric analysis of pediatric and nonhyperplastic prostate glands: evidence that BPH is not a unique stromal process. Prostate **33:** 177–182, 1997.

3. McNeal JE: Prostate, in Sterenberg SS (Ed): *Histology for Pathologists*, 2nd ed. Philadelphia, Lippincott-Raven, 1997, pp 997–1017.

4. Chagas MA, Babinski MA, Costa WS, *et al:* Stromal and acinar components of the transition zone in normal and hyperplastic human prostate. BJU Int **89:** 699–702, 2002.

5. Babinski MA, Chagas MA, Costa WS, *et al:* Prostatic epithelial and luminal area in the transition zone acini: morphometric analysis in normal and hyperplastic human prostate. BJU Int **92:** 592–596, 2003.

6. Bierhoff E, Walljaspes V, Hofmann D, *et al:* Morphological analogies of fetal prostate stroma and stromal nodules in BPH. Prostate **31:** 234–240, 1997.

7. Lepor H: Nonoperative management of benign prostatic hyperplasia. J Urol **141:** 1283–1289, 1989.

8. Ishigooka M, Hayami S, Hashimoto T, *et al:* Correlations between parameters in uroflowmetry and histological compositions in patients with benign prostatic hyperplasia. Urol Int **57:** 48–50, 1996.

9. Shapiro E, Haratanto V, and Lepor H: The response to alpha blockade in benign prostatic hyperplasia is related to the percent area density of prostate smooth muscle. Prostate **21:** 297–307, 1992.

10. Ichiyanagi O, Sasagawa I, Ishigooka M, *et al:* Relationship between urodynamic type of obstruction and histological component of the prostate in patients with benign prostatic hyperplasia. Eur Urol **36:** 203–206, 1999.

11. Ishigooka M, Hashimoto T, Hayami S, *et al:* Relative and total volume of histological components in benign prostate hyperplasia: relationship between histological components and clinical findings. Prostate **29:** 77–82, 1996.

12. Shapiro E, Becich MJ, Hartanto V, *et al:* The relative proportion of stromal and epithelial hyperplasia is related to the development of symptomatic benign prostatic hyperplasia. J Urol **147:** 1293–1297, 1992.

13. Bartsch G, Muller HR, Oberholzer M, *et al:* Light stereological analysis of the normal human prostate and of benign prostatic hyperplasia. J Urol **122:** 487–491, 1979.

14. Deering RE, Bigler SA, King J, *et al:* Morphometric quantitation of stroma in human benign prostatic hyperplasia. Urology **44:** 64–70, 1994.

15. McNeal JE: Pathology of benign prostatic hyperplasia. Urol Clin North Am **17:** 477–486, 1990.

16. Mattfeldt T, Mobius HJ, and Mall G: Orthogonal triplet probes: an efficient method for unbiased estimation of length and surface of objects with unknown orientation in space. J Microsc **139:** 279–289, 1985.

17. Weibel ER, Kistler GS, and Scherle WF: Practical stereological methods for morphometric cytology. J Cell Biol **30:** 23–38, 1966.

18. Gundersen HJG, Bendtsen TF, Korbo L, *et al:* Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS **96:** 379–394, 1988.

19. Pinheiro ACAD, Costa WS, Cardoso LEM, *et al:* Organization and relative content of smooth muscle cells and extracellular matrix components in the corpus cavernosum of the rat penis. J Urol **164:** 1802–1806, 2000.

20. Ichiyanagi O, Sasagawa I, Ishigooka M, *et al:* Morphometric analysis of symptomatic benign prostatic hyperplasia with and without bladder outlet obstruction. Urol Res **28:** 29–32, 2000.

21. Haralson MA, and Hanssel JR: The extracellular matrix—an overview, in Haralson MA, and Hanssel JR (Eds): *Extracellular Matrix: A Practical Approach*. Oxford, Oxford University Press, 1995, pp 1–20.

22. Marks LS, Treiger B, Dorey FJ, *et al:* Morphometry of the prostate: I. Distribution of tissue components in hyperplastic glands. Urology **44:** 486–492, 1994.

23. Lepor H, Tang R, and Shapiro E: The alpha-adrenoceptor subtype mediating the tension of human prostatic smooth muscle. Prostate **22:** 301–307, 1993.

24. Lepor H, Tang R, Meretyk S, *et al:* Binding and functional properties of alpha-1 adrenoceptors in different regions of the human prostate. J Urol **150:** 253–256, 1993.

25. McConnell JD: Medical management of benign prostatic hyperplasia with androgen suppression. Prostate Suppl **3:** 49–59, 1990.

26. Pradhan BK, and Chandra K: Morphogenesis of nodular hyperplasia—prostate. J Urol **113:** 210–213, 1975.

27. Cardoso LEM, Costa WS, and Sampaio FJ: Stromal modifications in benign prostatic hyperplasia as evidenced by glycosaminoglycan composition. Int Braz J Urol **26:** 630–634, 2000.

28. Hay E: *Cell Biology of Extracellular Matrix,* 2nd ed. New York, Plenum Press, 1991.

29. Carvalho HF, and Line SRP: Basement membrane associated changes in the rat ventral prostate following castration. Cell Biol Int **20:** 809–819, 1997.

30. Shapiro E, Hartanto V, and Lepor H: Anti-desmin vs anti-actin for quantifying the area density of prostatic smooth muscle. Prostate **20:** 259–267, 1992.