STEROEOLOGICAL ANALYSIS OF ANDROGEN RECEPTORS IN PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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Summary

Introduction. Through androgen receptors, androgens regulate prostate cellular growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity, as well as development and progression of prostate cancer. Prostate cancer, and its primary glandular tissue are influenced by hormones, and it is used for therapeutic purposes. Anti-androgen treatment is carried out in patients with metastatic prostate cancer, in order to block effects of androgens. Immunohistochemical analysis of androgen receptors in the prostate cancer tissue may help us to assume how the tumors will react to the anti-androgen therapy, if they are androgen-positive, -negative, or hormone resistant tumors. Knowledge of the presence of androgen receptors in the tumor tissue may be a prognostic indicator in histopathological analysis. The aim of this study was stereological evaluation of androgen receptor expression in patients with benign prostatic hyperplasia and in patients with prostate cancer, before therapy. Material and Methods. Immunohistochemical analysis was carried out using anti-human androgen receptor monoclonal antibody 441. The presence and intensity of the androgen receptors were evaluated in 195 patients: 165 with benign prostatic hyperplasia and 30 with prostate cancer using Weibel’s multi-purpose M 42 stereological test system. Material was obtained by needle biopsy or transurethral resection of the prostate. Results. All secretary cells in patients with benign prostatic hyperplasia were androgen positive, while in patients with prostate cancer, all tumors were mostly androgen positive, some with foci of negativity. The resulting negative correlation with Gleason score and International Society of Urological Pathology grade was not statistically significant. Conclusion. Study results of stereological analysis of androgen receptors indicate that prior the therapy prostate cancer is androgen-dependent, with a high level of androgen receptor expression, although slightly lower compared to benign prostatic hyperplasia. Key words: Prostatic Neoplasms; Receptors, Androgen; Prostatic Hyperplasia; Immunohistochemistry; Pathology

Sažetak

Uvod. Androgeni preko androgenih receptorova utiču na čeljski rast i funkciju, proliferaciju, diferencijaciju, apoptozu, lipidni metabolizam i sekretornu aktivnost prostate i na razvoj i progresiju karcinoma prostate. Karcinom prostate, kao i njegovo primarno žlezdnato tkivo je u području hormona i ta činjenica je iskorišćena u terapijskim svrhem. Antiandrogena terapija se sprovede kod pacijenata sa metastatskim karcinomom prostate, upravo sa ciljem da blokira dejstvo androgena. Imunohistohemijskom analizom androgenih receptorova u tkivu prostate i karcinomom prostate, možemo da pretpostavimo kako će tumor reagovati na datu antiandrogenu terapiju, o drugim mjestima li su andro- gen- pozitivni i androgen-negativni, ili hormonski rezistentni tumori. Saznanja o zastupljenosti androgenih receptorova u tkivu tumora, mogu da budu pomoćni kao prognozički indikator, u patohistološkoj analizi. Cilj ove studije je stereološka evaluacija ekspresije androgenih receptorova kod pacijenata sa karcinomom prostate i benignom hiperplazijom, pre sprovedene terapije. Materijal i metode. Imunohistohemijska analiza je sprovedena upotrebom monoklonalnog androgen-receptora 441 antitela. Stereološki je procenjivano prisustvo i intenzitet androgenih receptorova kod 195 pacijenata sa benignom hiperplazijom (165) i karcinomom prostate (30), u metodu multinamenskog Vajbelovog (Weiβ) testnog sistema M-42. Materijal je dobijen biopsijom iglom ili transuretralnom resekcijom prostate. Rezultati. Sve sekretorne čelije kod pacijenata sa benignom hiperplazijom su bile androgen- pozitivne, dok su kod pacijenata sa karcinomom prostate, svi tumori mahom bili androgen- pozitivni, ali pojedini s fokusima negativnosti. Dobjena je negativna korelacija sa Gleasonovim skorom i International Society of Urological Pathology gradom grupom, koja nije statistički značajna. Zaključak. Rezultati studije stereološke analize androgenih receptorova ukazuju da je karcinom prostate pre terapije androgen- zavisan; ima visok stepen ekspresije androgenih receptorova, mada nešto manji u odnosu na benignu hiperplaziju. Ključne reči: neoplazme prostate; androgeni receptori; hiperplazija prostate; imunohistohemijska: patologija

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Androgen Receptors in Prostate Cancer and BPH

Abbreviations

AR – androgen receptor
PCA – prostate cancer
BPH – benign prostatic hyperplasia
TURP – transurethral resection of the prostate
ISUP – Integrated Quantitative Gleason Score
Vv – volume density
DHT – 5α-dihydrotestosterone
LHRH – luteinizing-hormone-releasing hormone
IHC – immunohistochemical staining
HE – hematoxylin-eosin
Pt – total number of points in the test system
Pf – total number of points placed on the section
Vvep C – volume density of the epithelium in carcinoma
Vvl C – volume density of lumen in carcinoma
Vvs C – volume density of stroma in carcinoma
Vvep H – volume density of epithelium in hyperplasia
Vvl H – volume density of lumen in hyperplasia
Vvs H – volume density of stroma in hyperplasia
Vvj C – volume density of all nuclei in carcinoma
Vvj þC – volume density of nuclei in carcinoma positive for AR
Vvj C – volume density of nuclei in carcinoma negative for AR
Vvep C – volume density of other histological elements in carcinoma
Vvj H – volume density of all nuclei in hyperplasia
Vvj þH – volume density of nuclei in hyperplasia positive for AR
Vvj H – volume density of nuclei in hyperplasia negative for AR
Vvo H – volume density of other histological elements in hyperplasia
GG – grade group

Introduction

Stereology is a multidisciplinary scientific field that uses two-dimensional sections of bodies or tissues quantitatively, and interprets their inner three-dimensional structure. For stereological measuring of different structures, various test systems are used [1, 2]. There are numerous parameters that can be determined by stereological measuring. Evaluation of volume density (Vv) has its advantages in ease of performance and the fact that it is a relative stereological unit that shows what part of the total area belongs to the studied phase (volumetric representation of the studied phase within a certain area). Using mathematical calculations, it was established that Vv can be determined from the density of points (Pt) that belong to the tested phase. The Vv is without dimension (mm<sup>2</sup>) and if we multiply the obtained values by 100, the value can be expressed in percentages. In this paper, values are presented in percentages. Values of Vv do not depend on the tissue arrangement (anisotropic or isotropic tissue), nor on technical characteristics and power magnification of the microscope, so correction coefficient is not required [1–4].

Prostate, as an accessory gland, is under the influence of hormones, but until today there have been no findings whether accessory sex glands, such as prostate, secrete hormones [5, 6]. Through androgen receptors (ARs) of the prostate, testicular androgens regulate vital aspects of the prostate, such as cellular growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity. Primary hormonal mediator of benign prostatic hyperplasia (BPH) is 5α-dihydrotestosterone (DHT). This androgen is the main intracellular metabolite of testosterone. It is produced focally in stromal cells from the circulating testosterone, under the influence of the enzyme 5-reductase. The DHT influences stromal cells autocrinally, and epithelial cells paracrinally, increasing their mitotic activity due to binding to the receptors in these cells. The mitotic effect of DHT is about ten times stronger than of testosterone. In addition to DHT, other factors can also influence the mitotic activity in the prostate, i.e. the concentration of estradiol. The effect of estradiol is based on the increase in the number of nuclear receptors for DHT in prostate cells [7].

The BPH is the most common disease in adult men. Prostate cancer (PCA) is one of the most common malignancies and the second leading cause of death among men in industrially developed countries. The development and progression of PCA and its primary glandular tissue, depend on testosterone and dihydrotestosterone. Back in 1941, Huggins and Hodges showed that PCA is under hormonal influence of androgens [8].

Transrectal needle biopsy is the gold standard in the pathohistological diagnosis of PCA, as well as the analysis of the prostatic tissue after transurethral resection of the prostate (TURP) and prostatectomy. Modern approach to PCA therapy is carried out according to the indications for each stage of the disease separately (monitoring, curative treatment and hormonal therapy) [9, 10]. Endocrine, adjuvant hormone therapy has a goal of inhibiting stimulatory actions of androgens on prostate carcinoma cells. This can be achieved by surgical or pharmacological castration. Administration of luteinizing hormone-releasing hormone (LHRH) agonists and/or anti-androgen leads to a pharmacological block-

Graph 1. Mean values of volume densities of epithelium (Vvep H, Vvep C), lumen (Vvl H, Vvl C) and stroma (Vvs H, Vvs C) in patients with benign hyperplasia and patients with prostate carcinoma

Grafikon 1. Srednje vrednosti volumenske gustine epite-
lja (Vvep H, Vvep C), lumenja (Vvl H, Vvl C) i strome (Vvs H, Vvs C) kod pacijenata sa benignom hiperplazi-
jom i karcinomom prostate
Hormone therapy is used to treat metastatic prostate cancer [11, 12]. Using immunohistochemical determination of ARs in patients with PCa, we wished to substantiate the claims that the majority of tumors are androgen-dependent from the beginning, and that the initial anti-androgen therapy is useful. Over time, therapies create clones of androgen resistant cells, which leads to the resistance of the tumor to therapy, to the androgen blockade, both morphologically and immunohistochemically, which prospectively could be proven by the analysis of the material gained by TURP or biopsy, but only in patients who did not undergo prostatectomy. This claim has also been presented by many other authors [13–15].

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Using pathohistological analysis after immunohistochemical staining (IHC) ARs were intranuclearly localized, and their determination could prospectively aid as a prognostic indicator for patients with metastatic PCa [16–18].

Material and Methods

The prospective/retrospective study was carried out at the Centre for Pathology and Histology of the Clinical Centre of Vojvodina in Novi Sad, Republic of Serbia. After transrectal needle biopsies of the prostate and TURP performed at the Clinic for Urology, the bioptic materials of 195 male patients were histopathologically analyzed. The materials were fixed in 4% formalin, embedded in paraffin blocks, cut and stained in a standard way with hematoxylin-eosin (HE), and analyzed immunohistochemically for AR antibodies (DAKO Corp.).

Using histological analysis, patients were divided into two groups: experimental group with histopathologically diagnosed PCa (30 patients) and a control group with histopathologically diagnosed BPH (165 patients).

M-42 Weibel’s multipurpose test system was used for stereological determination of the Vv of the prostate slices in both groups, meaning that the total number of test points was 42. M-42 grid has 6 lines in 7 rows, 21 lines and 42 points in total. Lines of the system are parallel and distributed opposite each other. The grid was installed in the eyepiece of the light microscope Galen.

Stereological analysis was performed on 4 fields of view in 4 nonadjacent histological cuts. The first field of view was selected randomly, and the next three adjacent fields of view were selected according to the principles of stereology, or isotropic principle. According to the same isotropic principle, certain elements within a field of view were counted and eliminated.

During the measurement, the test system overlapped the desired field of view of the observed structure, that is, points of the test system fell on the observed slices of the test tissue. Within the field of view, the total number of points placed on the section (Pf) were counted. Number of points of one phase, e. g. nuclei positive to androgen receptors in PCa, from one field of view were added to other data.
gained from the remaining three fields of view. Then we added up all the hits of one phase in four histological cuts and divided the resultant sub-total by the total number of points in the test system (Pt), i.e. 168 (42 x 4 = 168, because there were four fields of view). In this way we obtained the necessary data for the calculation of the Vv of the Pf according to the well-known formula: \( Vv = \frac{Pf}{Pt} \) (Vv - volume density of the tested phase, Pf - total number of points placed on the section, and Pt - total number of points in the test system; number of points of the test system multiplied by the number of analyzed fields of view).

After calculating the Vv of tested phases of one biopsy sample, we determined mean values for both tested groups.

Using stereological analysis we calculated a total of 14 parameters, that we divided in two groups based on staining methods. Based on HE staining: Vv of the epithelium in carcinoma (Vvep C), Vv of lumen in carcinoma (Vvl C), Vv of stroma in carcinoma (Vvs C), Vv of epithelium in hyperplasia (Vvep H), Vv of lumen in hyperplasia (Vvl H), and Vv of stroma in hyperplasia (Vvs H). Based on immunohistochemical staining for ARs: Vv of all nuclei in carcinoma (Vvj C), Vv of nuclei in carcinoma positive for AR (Vvj+C), Vv of nuclei in carcinoma negative for AR (Vvj-C), Vv of other histological elements in carcinoma (Vvo C), Vv of all nuclei in hyperplasia (Vvj H), Vv of nuclei in hyperplasia positive for AR (Vvj+H), Vv of nuclei in hyperplasia negative for AR (Vvj-H), and Vv of other histological elements in hyperplasia (Vvo H). Stereological measurements were performed at 100 x magnification (10 oc. x 10 obj.).

Standard statistical analysis was performed using the computer program Origin, tables and figures using Word and Excel, with calculation of general statistical indicators: mean value, i.e. the arithmetic mean, mode (typical) value and the median, standard error and standard deviation, minimum and maximum values, T-test and correlation.

**Results**

The mean age of all (195) patients in both groups was 69.26 ± 0.46, the oldest being 89, and the youngest 51 years old, and the median age of 69. The mean age of patients with PCa was 68.97 ± 1.44, the oldest being 81, and the youngest 51 years old. Most patients with cancer were in the seventh decade of life. The mean age of patients with BPH was 69.3 ± 0.4, the oldest being 89, and the youngest 53 years old. Most patients with hyperplasia were in the sixth decade of life.

The mean values of Vv obtained by stereological measurements based on standard HE staining in BHP and PCa are shown in Graph 1. The Figure 1 shows prostate cancer “pattern” 2 and 3 (Figure 1A, Figure 1B), “pattern” 4 and 5 (Figure 1C, Figure 1D) benign hyperplasia.

The mean values of Vv of glandular epithelium in PCa (57%) were percentually higher than the same values in benign hyperplasia (39%), but there was no statistically significant difference in the degree of freedom (p < 0.05). The Vv of glandular lumen in PCa (9%) was numerically lower than in BHP (41%), and there was a statistically significant difference in the degree of freedom (p < 0.05). The Vv of stroma in PCa (34%) was numerically higher than in hyperplasia (20%), but there was no statistically significant difference in the degree of freedom (p < 0.05). Standard deviations (SDs) for Vv of the epithelium, glandular lumen and stroma in patients with BHP were 0.03, 0.01 and 0.03, respectively. In patients with PCa, the SDs for Vv of the epithelium, glandular lumen and stroma were 0.05, 0.06 and 0.04, respectively.

By comparing Integrated Quantitative Gleason Score (ISUP) grade group (GG) and Vv of histological elements in prostate cancer based on HE staining, the following results were obtained: between GG and Vv of the epithelium there was a correlation (0.11), positive and insignificant; between GG and Vv of the lumen there was a correlation (-0.47), negative and low; between GG and Vv...
Androgen-pozitivna jedra sekretornih ćelija i -

of the stroma there was also a correlation (0.09), positive and insignificant. It can be concluded that the increase in tumor dedifferentiation causes an increase in the amount of epithelium and stroma, and a decreased lumen of adenoid structures.

We also analyzed the mean values of Vv by stereological measurements after immunohistochemical staining for AR, in patients with BHP and PCa (Graph 2, as well as Figure 2, and Figure 3).

All nuclei of glandular epithelium in BHP were positive for AR (Vv of all nuclei equaled Vv of AR+ nuclei). Carcinomas with certain number of nuclei that did not stain for ARs (AR-nuclear) were detected. The mean value of Vv of all nuclei in carcinoma was 40%, and was numerically higher than the Vv of all the nuclei in hyperplasia (20%), with the difference statistically significant in the degree of freedom (p < 0.05). The Vv of nuclei negative for AR in carcinoma was 5% and was numerically higher than the same Vv in hyperplasia (0%), but it was not statistically significant in the degree of freedom (p > 0.05). The Vv of other histological elements was numerically higher than in BHP, and the difference was statistically significant in the degree of freedom (p < 0.05). The SD for Vv of all nuclei, nuclei positive for AR, nuclei negative for AR and other histological elements in patients with PCa were as follows: 0.03; 0.02; 0.02 and 0.03.

The SD for Vv of all nuclei, nuclei positive for AR, nuclei negative for AR and other histological elements in patients with BPH were as follows: 0.03; 0.03; 0 and 0.03.

By comparing ISUP grading and Vv of histological elements in PCa based on IHC staining for AR, the following results were obtained: between GG and Vv of all nuclei there was a correlation (0.09), positive and insignificant; between GG and Vv of AR + nuclei there was a correlation (0.05), positive and insignificant; between GG and Vv of AR - nuclei there was a correlation (0.09), positive and insignificant; the correlation also existed between GG and Vv of other histological elements, negative and insignificant (-0.25). It can be concluded that increased tumor dedifferentiation caused an increase in the number of nuclei, and a decrease of other histological elements.

Discussion

Formation of two groups of patients, those with benign hyperplasia being the control group and those with PCa being the experimental group, enabled quantitative presentation of histological differences between the two conditions. For stereological measurements of structures such as prostatic parenchyma, a multipurpose test system is recommended, so we used and one of the most frequently used multi-purpose test systems, M-42. In this type of test the entire system has a total of 42 points, and the number of points that correspond to certain phases is divided by 42.

Hematoxylin-eosin staining determined the Vv of the epithelium, glands and stroma lumens, and immunohistochemical staining determined the Vv of: all nuclei, androgen positive, androgen negative nuclei, and other histological elements. The numerical difference between the Vv of epithelium, stroma and lumen between the control and the experimental group was examined, but it was not statistically significant for the degree of freedom (p < 0.05). The Vv of the epithelium and stroma in carcinoma was higher than in hyperplasia, unlike Vv of lumen which was lower, corresponding to morphological appearance of changes. Histomorphologically, in prostate cancer, multiplication of glandular, cribriform structures, and formation of chains, strips and solid zones that consist of atypical cells is associated with an increase of the Vv of the epithelium. The same occurs with stroma, for it is important for growth, support, i.e. nutrition of tumors. It can be argued that the increase in Vv of epithelium in carcinoma comes at the expense of reduction of Vv of lumens, and that it is more evident if the tumors are more dedifferentiated [19].

After immunohistochemical staining for AR, it can be concluded that all nuclei in BPH are androgen-dependent, i.e. Vv of all nuclei is equal to Vv of androgen-positive nuclei; however, in PCa there are androgen independent nuclei, which is consistent with literature data [19–24]. The obtained results are consistent with literature data that the majority of prostate adenocarcinomas are AR positive [13, 14, 19–22].

All 30 patients of the experimental group had a histomorphological diagnosis of acinar adenocarcinoma. Other histological types of PCa were not detected, as expected, considering that it accounts for more than 90% of all histological types of PCa [21].

Results of comparison between stereologically evaluated AR and Gleason score were negative with slight correlation (-0.12). Approximate values were obtained by comparing ISUP grade group and semi-
quantitatively evaluated AR (-0.15). It can be argued that the increase in Gleason score and ISUP grade group in certain number of carcinomas lead to the decrease in the number of nuclei with positive AR. The more dedifferentiated the tumor, the more likely it is to have androgen-resistant cells. However, it should not be left out that certain tumors of the same grade had differently evaluated AR, meaning that based only on morphology (HE staining), there is no way to determine the precise extent and intensity of nuclei positive for AR. Our results correlate with results of other authors, who claim that carcinomas with low scores do not have a significantly higher content of AR, than those with high Gleason score. On the other hand, some authors claim otherwise, but one cannot exclude studies that have not determined the existence of correlation between Gleason score and AR, which leaves space for staining of antibodies for AR [13–15, 23–33].

Conclusion

Stereologically calculated volume densities of histological elements in benign prostatic hyperplasia and prostate carcinoma are consistent with morphological appearance of lesions. Compared to benign hyperplasia, prostate carcinoma has higher volume density of the epithelium and stroma.

Volume density of androgen-sensitive nuclei is lower in carcinoma than in benign prostatic hyperplasia; more precisely, all secretory cells in benign prostatic hyperplasia are androgen-sensitive, while in carcinoma, there are also androgen-negative nuclei.

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