SHORT COMMUNICATION

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Immunohistochemical expression of androgen receptors in prostate carcinoma and benign prostatic hyperplasia

Imunohistohemijska ekspresija androgenih receptora kod karcinoma prostate i benigne hiperplazije prostate

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Abstract

Background/Aim. Prostate carcinoma (PCa) and its parent organ are influenced by hormones, which is used for therapeutic purposes. Through androgen receptors (AR) androgens influence cell growth and function, proliferation, differentiation, apoptosis, lipid metabolism and secretory activity of the prostate, as well as development and progression of PCa. An antiandrogen therapy is carried out in patients with metastatic PCa, in order to block effects of androgens. By conducting immunohistochemical analysis of androgen receptors in the PCa tissue, we can assume how the tumour will react to an administered antiandrogen therapy, both in androgen-positive and androgen-negative, resistant tumours. Knowledge of the presence of AR in the tumour tissue may serve as a prognostic indicator in histopathological analysis. The aim of this study was to evaluate the expression of AR in patients with benign prostatic hyperplasia (BPH) and in those with PCa, before therapy. **Methods.** Immunohistochemical analysis was carried out by using anti-human AR monoclonal antibody AR441 (DAKO), and presence and intensity of AR were semi-quantitatively evaluated in 195 patients, 165 with BPH and 30 with PCa. Material for analysis was obtained by needle biopsy or transurethral resection of the prostate (TURP). **Results.** All secretory cells in patients with BPH were intensively androgen positive, while in patients with PCa they were mostly moderately to highly positive, but with foci of negativity. The observed negative correlation between AR and Gleason score and the International Society of Urologic Pathology (ISUP) grade group of PCa was not statistically significant. **Conclusion.** Study results indicate that PCa, before therapy, is androgen-dependent, with a high level of AR expression.

Key words:
prostate neoplasms; receptors, androgen; prostatic hyperplasia; immunohistochemistry.

Apstrakt

Uvod/Cilj. Karcinom prostate (PCa), kao i njegov ishodišni organ se nalaze pod uticajem hormona, što je iskorišćeno u terapijske svrhe. Androgeni preko androgenih receptora (AR) utiču na ćelijski rast i funkciju, proliferaciju, diferencijaciju, apoptozu, lipidni metabolizam i sekretornu aktivnost prostate, ali i na razvoj i progresiju PCa. Antiandrogena terapija se sprovodi kod bolesnika sa metastaskim PCa, upravo sa ciljem da blokira dejstvo androgena. Imunohistohemijskom analizom AR u tkivu prostate sa karcinomom, možemo da pretpostavimo kako će tumor reagovati na datu antiandrogenu terapiju, bilo da se radi o androgen pozitivnim ili androgen negativnim, rezistentnim tumorima. Saznanja o zastupljenosti AR u tkivu tumora mogla bi poslužiti kao prognostički indikator u patohistološkoj analizi. Cilj rada je bio evaluacija ekspresije AR kod bolesnika sa benignom hiperplazijom (BHP) i kod bolesnika sa PCa, pre sprovedene terapije. **Metode.** Imunohistohemijska analiza je sprovedena uz upotrebu *anti-human* AR *monoclonal antibody* AR441 (DAKO), uz semikvantitativnu procenu prisustva i inteziteta AR kod 195 bolesnika, 165 sa BHP i 30 sa PCa. Materijal je dobijen iglenom biopsijom ili transuretralnom resekcijom prostate (TURP). **Rezultati.** Sve sekretorne ćelije kod bolesnika sa BHP su bile intezivno androgen pozitivne, dok su kod bolesnika sa PCa, mahom bile umereno do izrazito pozitivne, ali sa fokusima negativnosti. Uočena je negativna korelacija AR sa Gleason skorom i *International Society of Urologic Pathology* (ISUP) gradus grupom PCa, koja nije bila statistički značajna. **Zaključak.** Rezultati studije su pokazali da je PCa, pre sprovedene terapije, androgen zavisan sa visokim stepenom ekspresije AR.

Ključne reči:
prostata, neoplazme; receptori, androidni; prostata, hiperplazija; imunohistohemija.

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Introduction

So far, there have been no findings whether accessory sex glands, such as prostate, secrete hormones, but it was proved that they are under the influence of hormones [1, 2]. Through androgen receptors (AR) in prostate tissue testicular androgens regulate vital aspects of the gland, such as: cell 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, and it is produced focally in stromal cells from the circulating testosterone, under the influence of the enzyme 5-reductase. DHT influences stromal cells autocrinally, and epithelial cells paracrinally, increasing their mitotic activity due to binding to receptors in these cells. Mitotic effect of DHT is about ten times stronger than the same effect of testosterone. In addition to DHT, other factors can also influence the mitotic activity in the prostate, such as the concentration of estradiol. The effect of estradiol is based on the increase in the number of nuclear receptors for DHT in prostate cells [3].

Benign prostatic hyperplasia is the most common disease of this gland in men, and PCa is one of the most diagnosed malignancies and the second leading cause of death among men in industrialised countries. The development and progression of PCa, as well as its parent tissue, depend on testosterone and dihydrotestosterone. Back in 1941, Huggins and Hodges [4] stated the assumption that PCa is under hormonal influence of androgens.

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) [5, 6]. The endocrine, hormone therapy is used to cure metastatic carcinoma. It acts adjuvantly with a goal to inhibit stimulatory actions of androgens on PCa cells. This can also be achieved by surgical or pharmacological castration. Administration of gonadotropin-releasing hormone (LH-RH) agonists and/or antiandrogen leads to a pharmacological blockade [7].

Prostate carcinoma therapy is preceded by its diagnostics, wherein a pathologist has the final decision. The gold standard for the histopathological diagnosis of PCa is prostate biopsy, as well as the analysis of the prostatic tissue after transurethral resection of the prostate (TURP) and prostatectomy.

Using immunohistochemical determination of AR in patients with PCa we wish to morphologically substantiate the claims that the majority of tumours is androgen-dependent from the beginning, and that the initial antiandrogen therapy is purposeful. Over time, therapies create clones of androgen resistant cells, which leads to the resistance of the tumour to the androgen blockade, which prospectively, could morphologically and immunohistochemically be proven by the analysis of the material gained by TURP or prostate biopsy, of course only in patients who did not undergo prostatectomy. This claim has also been presented by many other authors [8–10].

Histopathological analysis after immunohistochemical staining (IHC) has revealed that AR are intranuclearly located, and their determination could prospectively serve as a prognostic indicator for patients with metastatic PCa [11–14].

Methods

The study was prospective and retrospective, and was carried out in the Centre for Pathology and Histology of the Clinical Centre of Vojvodina in Novi Sad, Republic of Serbia, wherein the materials of 195 male patients were histopathologically analyzed, after being obtained by transrectal needle biopsies of the prostate tissue and TURP at the Clinic for Urology. The materials were fixed in 4% formalin, and then they were embedded in paraffin blocks, cut and stained in a standard way, with hematoxylin-eosin (HE), and analyzed immunohistochemically for androgen receptor antibodies (DAKO).

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

After immunohistochemical staining, AR of secretory cells were semi-quantitatively evaluated. Negatively stained nuclei were marked with a zero (0), and positively stained nuclei with a plus (+). The intensity of nuclei staining was also evaluated as follows: light staining (+), moderate staining (++), and pronounced nuclei staining (+++).

In addition to the immunohistochemical analysis of AR, age and prostate specific antigen (PSA) levels were analyzed in both groups of patients, with additional analysis of the Gleason score and the International Society of Urological Pathology (ISUP) grade group in patients with PCa.

Results

Mean age of all 195 patients in both groups was 69.26 ± 0.46 years (median 69 years), with the oldest being 89, and the youngest 51 years old. Mean age of patients with PCa was 68.97 ± 1.44 years, with the oldest being 81, and the youngest 51 years old. Most patients were in the seventh decade of life. Mean age of patients with BPH was 69.3 ± 0.4 years, with the oldest being 89, and the youngest 53 years old. Most patients were in the sixth decade of life. Difference in patients' age comparing the experimental and the control group was not wss not statistically significant.

Mean serum PSA level in all 195 patients was 15.97 ± 1.58 ng/mL, the lowest measured value being 1.62 ng/mL, the highest 115 ng/mL, and the most frequent one was 11.15 ng/mL. Mean value of PSA levels in PCa patients was 21.64 ± 4.5 ng/mL. The lowest measured value was 5.8 ng/mL, and the highest one 115 ng/mL. Mean value of PSA levels in BPH patients was 14.16 ± 1.46 ng/mL. The lowest measured value was 1.62 ng/mL, and the highest one 110 ng/mL. There was no statistically significant difference between PSA levels in the two study groups.

Table 1 shows the distribution of patients according to the semi-quantitatively estimated values of AR in PCa, compared to the number of patients. Using the semi-quantitative analysis of androgen receptors, tumours were given 2.7 ± 0.1 pluses on average, with the minimum of two, maximum of three, and most frequently three pluses. Most carcinoma patients (21 people or 70%) have the value of AR (+++), and the remaining patients (9 people or 30%) the value of AR (++). There were no patients whose tumour AR were semi-quantitatively graded with (+) or zero (Figures 1a–f).

Table 1
Distribution of patients with prostate carcinoma (PCa) according to the semi-quantitatively determined representation of androgen receptors (AR) in the PCa tissue

Semi-quantitative evaluation of androgen receptors	Patients, n (%)
0	0 (0)
+	0 (0)
++	9 (30)
+++	21 (70)
Total	30 (100)

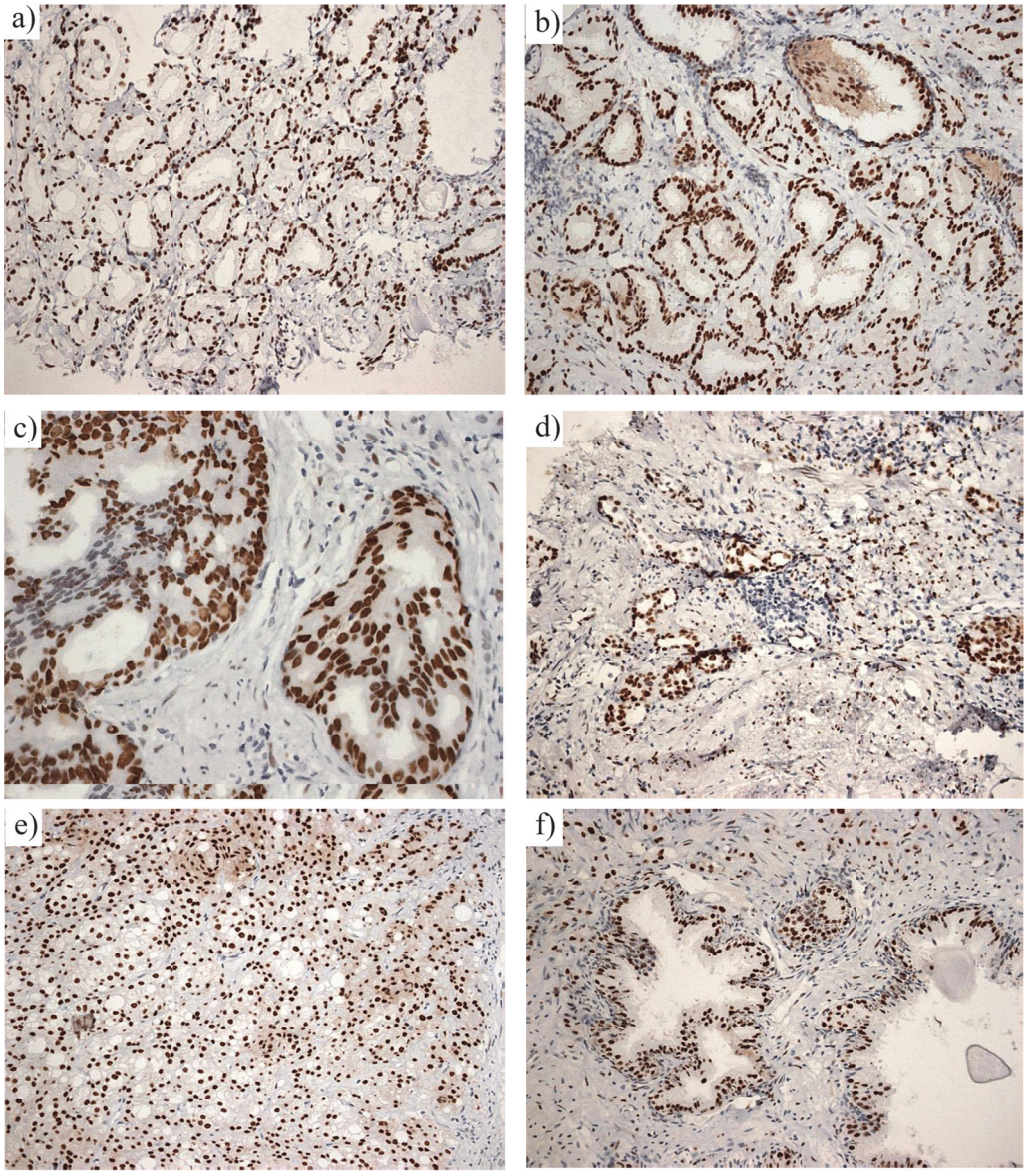

Fig. 1 – Immunohistochemical staining for androgen receptors in the prostate tissue: a) Androgen positive nuclei of prostate adenocarcinoma tumour cells (+++), the Gleason grade 2 (×20); b) Androgen positive nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 3 (+++) and androgen negative nuclei of *PIN basal cell layer* (×20); c) Androgen positive and androgen negative nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 3 (++), (×40); d) Androgen positive and androgen negative nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 4 (++) (×20); e) Androgen positive nuclei of prostate adenocarcinoma tumour cells, the Gleason grade 5 (+++) (×20); f) Androgen negative nuclei of basal cell layer and androgen positive nuclei of secretory cells of the prostate gland with benign prostatic hyperplasia (×20).

By comparing the semi-quantitatively estimated AR with the Gleason score and the ISUP grade group it could be noticed that with the increase of the Gleason score, tumour dedifferentiation, the number of nuclei with positive AR in carcinoma tissue decreased. The correlation existed, it was negative, but slight (-0.125). By comparing the semi-quantitatively estimated AR and the ISUP grade group it could also be noticed that with an increase in histological grade, tumour dedifferentiation, the number of AR+ nuclei in carcinoma decreased. The correlation existed, it was negative, but slight (-0.16). Intensity of nuclei staining for AR was identical in all tumours being (+++).

The semi-quantitative evaluation of AR in patients with BPH (the control group) showed that all nuclei of glandular epithelium secretory cells were AR positive (+++). Basal cell nuclei were AR negative (0) (Figure 1f).

Discussion

Benign prostatic hyperplasia is the most common prostate gland disease [15], as evidenced by our study with 30 (15.38%) individuals having carcinoma, and 165 (84.62%) having BPH.

Mean age of people with carcinoma in our study was 68.97 ± 1.43 years, with the youngest being 51, and the oldest 81 years old, and most patients (14 patients or 46.66%) were in the seventh decade of life. Our results are consistent with the literature, and they indicate that PCa is a disease of men older than 50 years and that only 1% of these tumours are diagnosed in people under 50, and that their incidence reach the peak around the age of 75 [15].

All 30 patients of the experimental group (with PCa) had a histomorphological diagnosis of acinar adenocarcinoma, with no other types of PCa detected, as was expected, considering it accounts for over 90% of all histological types of PCa [16, 17].

The semi-quantitaive evaluation of AR in carcinoma patients evaluated tumours with 2.7 ± 0.1 pluses on average, with the minimum of (++) and maximum of (+++), most often (+++). More than two thirds of patients with carcinoma (21 people or 70%) were evaluated as AR (+++), and about one third (9 people or 30%) as AR (++). Among the patients there were no those whose AR were semi-quantitatively evaluated with (+) or (0). The results obtained are in accordance with literature data that the majority of prostate adenocarcinomas has positive AR [18–20].

Results of correlation of semi-quantitatively evaluated AR with the Gleason score were negative, with negligible correlation coefficient (-0.125). Approximate values were obtained by correlating the ISUP grade group and semi-quantitatively evaluated AR (-0.16). It can be argued that in certain number of carcinoma the increase in the Gleason score and the grade group causes the decrease in the number of nuclei with positive AR. The more dedifferentiated tumour, the more likely it will have androgen-resistant cells. However, it should not be left out that certain tumours of the same grade had differently evaluated AR, meaning that only morphology (hematoxylin-eosin staining) fails to show the precise extent and intensity of nuclei positive for AR. Our results correlate with the 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 side, there are the authors who claim otherwise, but one cannot exclude studies that have not determined the existence of correlation between the Gleason score and AR representation in the PCa tissue [21–30].

Conclusion

All analysed tumours were androgen sensitive (+++ or ++).

Prostate carcinomas of the same Gleason score or ISUP grade group had different degree of AR presence, from which follows that based only on histomorphological appearance of carcinoma, its ISUP grade group or the Gleason score on HE staining, the extent and intensity of nuclei positive for AR cannot be precisely determined.

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