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Assessment of Immunohistochemical Expression of Androgen Receptor and its Correlation with Histopathological Prognostic Factors in Prostatic Carcinoma

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Abstract

Background: **Prostate carcinoma (PCa) is the second most commonly diagnosed tumor in men worldwide and is the most common male malignancy in developed countries. Androgens play a main role in the growth, differentiation, and maintenance of prostate tissue via Androgen Receptor (AR). AR expression represents a potential prognostic marker for prostatic carcinoma.** ***Aim:*** **this study aims to assess of immunohistochemical expression of the Androgen receptor and its correlation with histopathological prognostic factors in Prostatic carcinoma.** ***Methods:*** **A cross-section analytical study was conducted at the pathology department of the Faculty of Medicine at Suez Canal University. Thirty-five prostatic specimens with prostate cancer were analyzed for immunohistochemichal expression of the Androgen receptor [AR (A19611)].** ***Results:*** **Out of the studied thirty-five specimens. There is a statistically significant difference between different grade groups regarding AR expression (P value =0.008) & there is a statistically significant difference between different Gleason scores regarding AR expression (P value =0.001). There is no statistically significant difference between AR expression and Age of patients, Perineural invasion, multicenticity, and no benign prostatic changes.** ***Conclusions:*** **This study focused on evaluating the relationship of Androgen Receptor expression with various prognostic factors associated with carcinoma prostate, AR was upregulated in prostate cancer; mainly in cancers with a worse prognosis which has a higher Gleason Score and higher-grade group.**

Keywords: Androgen receptor, immunohistochemistry, prostatic adenocarcinoma.

Introduction

Prostatic carcinoma is a disease of increasing significance and is the 2nd most commonly diagnosed tumor in men worldwide and is the most common male malignancy in developed countries[1], with the highest incidence between 70 and 75 years old. Prostatic carcinoma is a growing concern in Egypt and currently ranks as the 4th most common cancer in the country with an incidence rate of about 4.5% among male cancer patients[2]. There are several risk factors affecting PCa, some of which are nonmodifiable

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(including age, race/ethnicity, family history, and Geography), while others are modifiable (including diet, obesity, smoking, chemical exposures, prostatic inflammation, sexually transmitted infections, and vasectomy)[3]. Prostatic carcinoma is a heterogeneous disease process with a various spectrum of light microscopic morphologic as well as biological characteristics. Diagnosis of prostate malignant tissue can sometimes present a diagnostic challenge for pathologists since some forms of benign prostate lesions can mimic PCa. band the architectural or cytologic clues for the diagnosis of carcinoma may not usually be seen in small foci of suspicious glands[4]. Histopathological diagnosis of PCa can be done by transrectal ultrasound-guided (TRUS) biopsy[5] after an abnormal finding on digital rectal examination or finding a rise in prostate-specific antigen (PSA) level[6]. Androgens play a fundamental role in the growth, differentiation, and maintenance of prostate tissue. Their effects are mediated via a specific androgen receptor (AR) that belongs to the nuclear receptor family[7]. The AR molecule is a main part of the regulatory androgen-AR complex and is therefore critical in the androgen-AR pathway of PCa[8,9]. It is believed that prostatic carcinogenesis is an androgen-mediated tumor, however, serum androgens can NOT induce carcinogenesis alone, thus the functional status of the androgen receptor (AR) is the most important mediator of prostate cancer progression. Low serum testosterone in prostate cancer patients was found to be associated with high AR expression which in turn is associated with higher Gleason score. Some studies also showed that high AR expression was correlated with disease progression and a lower recurrence-free survival rate[10]. Hence in the current study, we aimed to assess of immunohistochemical expression of the Androgen receptor and its association with various histopatthological prognostic parameters like tumor quantification, Gleason scoring, WHO grade group, and perineural invasion. Our work aimed to assess the expression patterns of Androgen Receptors in prostatic adenocarcinoma in correlation with the histopathological findings to evaluate its role as a prognostic marker or possible therapeutic target.

Materials and Methods

This cross-sectional analytic study included formalin-fixed, paraffin-embedded blocks of prostatectomy, TURP (Transurethral resection of the prostate), and TRUS (Transrectal ultrasound) specimens diagnosed as prostatic adenocarcinoma archived in the pathology laboratory, Suez Canal University Hospital during the period from January 2011 to December 2019. The required clinico-pathological data was obtained from medical records. One slide was re-cut from each block stained by Haematoxylin and Eosin (H&E) and re-examined to confirm the diagnosis. Sections from each block were cut at 5-µm-thickness and prepared for Immunohistochemical staining for AR. Sections were placed onto positively charged slides, heat-induced epitope retrieval was done in a microwave, and the prepared primary antibody of AR (A19611) from Novus Biomedical was used according to the steps mentioned in the company datasheet. By using light microscopy, immunohistochemically stained tissue

sections were examined at high power magnification, and the nuclear staining percentage for the marker was calculated semi-quantitatively and compared to the positive and negative controls. Immunohistochemical expression of AR is interpreted via Allred score. The Allred score is the sum of adding intensity and the proportion scores[11]. The intensity of staining (IS) is scored on a scale of from 0 to 3 as follows: 0=no staining,1=weak staining, 2=moderate staining, and 3=strong staining. The proportion of positive cells is scored on a scale from 0 to 5 as follows the proportion score (PS): 0=0%, 1=<1%, =1–10%, 3=11–33%, 4=34–66%, and 5=67–100%. Finally, the scores for both the staining intensity and the proportion of cells are summed to get a final total score (TS) of 0–8 which is given as TS = PS + IS. TS=0 and 2 are negative scores TS =3, 4, 5, 6, 7, and 8 are positive scores[12]. The immunohistochemical findings will be correlated with the H&E findings.

Statistical Analysis

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**)** Qualitative data were described using numbers and percentages. The Kolmogorov-Smirnov test was used to verify the normality of distribution Quantitative data were described using range (minimum and maximum), mean, standard deviation, median, and interquartile range (IQR). The significance of the obtained results **was** judged at the 5% level. The chi-square test was used for categorical variables, to compare different groups. Fisher's Exact or Monte Carlo correction was used for correction for chi-square when more than 20% of the cells have an expected count of less than 5. Student t-test was used for normally distributed quantitative variables, to compare between two studied groups. and Kruskal Wallis test or abnormally distributed quantitative variables, to compare between more than two studied groups.

Results

Demographic data of patients

Out of the studied thirty-five specimens, specimens were divided into 3 groups: <50 years, 50-70 years & >70 years. 19 specimens (54.3%) were at age group (>70),13 specimens (37.1%) were at age group (50-70) and 3 specimens (8.6%) were at age group (<50).

Histopathological assessment

Histopathological examination of the examined specimens showed total Gleason score ranged from 6 to 9, with Mean score (7.49), where 4 specimens (11.4%) had total Gleason score (6), 14 specimens (40.0%) had total Gleason score (7), 13 specimens (37.1%) had total Gleason score (8) and 4 specimens (11.4%) had total Gleason score (9). By assessment of Grade Group distribution, 4 specimens (11.4%) were in grade group I, 5 specimens (14.3%) were in Grade Group II, 9 specimens (25.7%) were in grade group III, 13 specimens (37.1%) were grade group IV, and 4 specimens (11.4%) were at grade group V.

Immunohistochemical assessment

According to the Allred score 30 specimens (representing 85.7% of all specimens) showed positive nuclear staining of AR (total score=3-8) while 5

specimens (20.8%) showed negative nuclear staining (total score=0-2) with a mean score (4.19). Of the 30 AR-positive specimens, 7 specimens (23.33%) showed strong staining, 16 specimens (53.33%) showed moderate staining and 7 specimens (23.33%) showed weak staining. Specimens were divided into two groups according to Allred score (positive and negative) and then compared with different histopathological parameters: Correlation between Gleason scores and AR expression: Of the 30 AR positive specimens, 1 specimen (representing 3.3%) was total Gleason score (6), 12 specimens (representing 40.0%) were total Gleason score (7), 13 specimens (representing 43.3%) were total Gleason score (8) and 4 specimens (representing 13.3%) were total Gleason score (9), with mean score (7.67). Of the 5 AR negative specimens, 3 specimens (60.0%) had a total Gleason score (6) and 2 specimens (20.0%) had a total Gleason score (7), No specimens scored (8) or (9) with a mean score of 6.40. Based on the above-mentioned results, there is a statistically significant difference between Gleason scoring regarding AR expression with (P value =0.008). (Figure 1).

Correlation between different grade groups and AR expression:

Of the 30 AR positive specimens;1 specimen (3.3%) was grade group I, 3 specimens (10.0%) were grade group II, 9 specimens (30.0%) were grade group III, 13 specimens (43.3%) were grade group IV while 4 specimens (13.3%) were grade group V. Of the 5 AR negative specimens, 3 specimens were grade group I (60.0%), 2 specimens were grade group II (40.0%) while none of the negative specimens were grade group III, IV, or V. Based on the above-mentioned results, there is a statistically significant difference between Gleason scoring regarding AR expression with (P=0.001). (Figure 2).

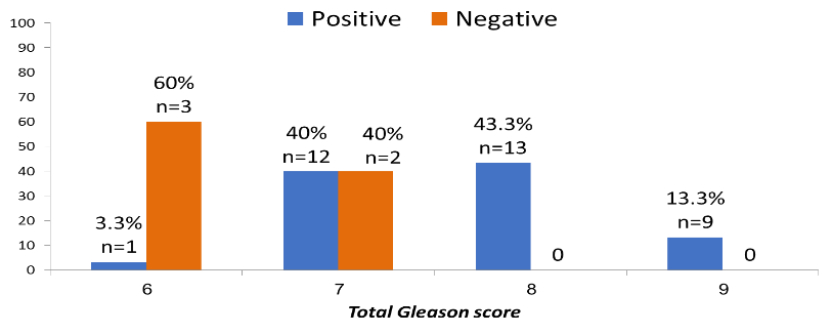

Figure 1: Correlation between AR scoring and Total Gleason score.

There was no statistically significant correlation between AR expression and demographic data of patients or other histopathological parameters including :Tumor burden in tissues, multicentricity, benign changes, and perineural invasion. AR expression in stromal cells was not recorded.

Discussion

Prostate cancer is the most common cancer diagnosed in men, with nearly 410,000 diagnoses in Europe yearly. Approximately 20–25% will develop metastatic disease, which progresses

to lethal castration-resistant prostate cancer (CRPC)[13]. AR in epithelial cells of prostatic carcinoma was studied by several authors[13-15]. Some authors found higher expression of AR in epithelial cells in well-differentiated tumors compared to moderately and poorly differentiated lesions[16,17]. Androgens regulate the proliferation rates of epithelial cells, so increased androgen levels or AR activity could result in unlimited proliferation and cancer development. AR is required for normal prostate function and is expressed in prostatic intra-epithelial neoplasias (PIN) and early carcinoma; however, it is also expressed in advanced and metastatic carcinoma[18].

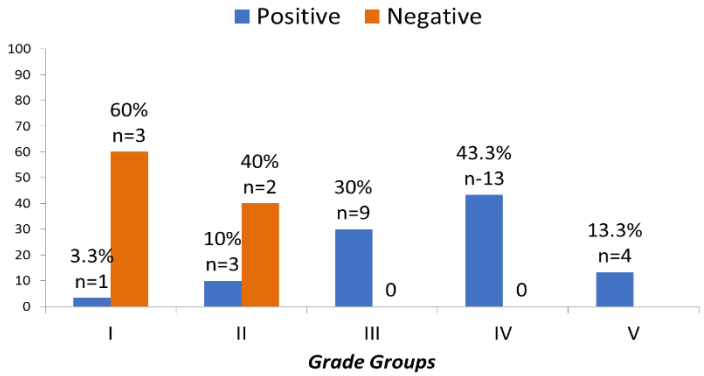

Figure 2: Correlation between AR scoring and different Grade Groups

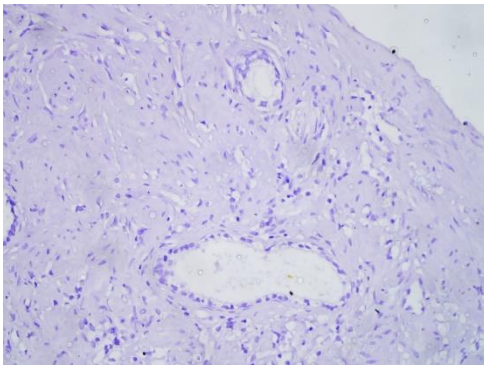

Figure 3: Representative photomicrographs of Negative AR immunostaining (400X)

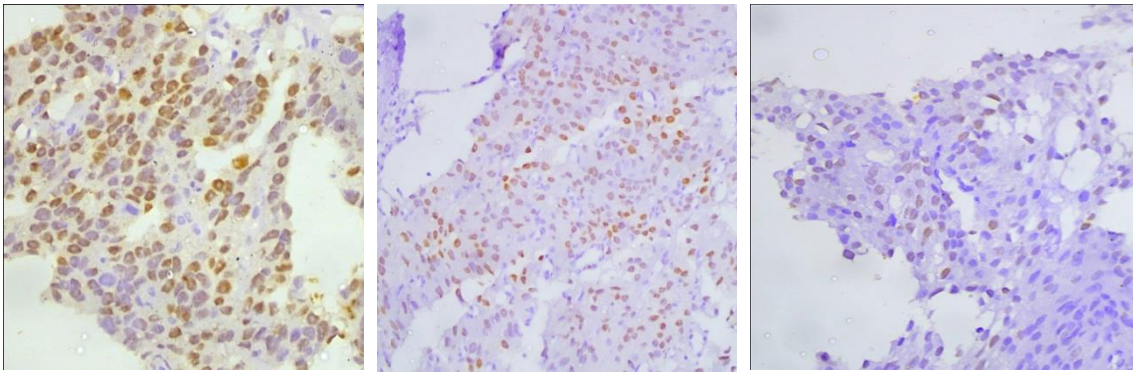

Figure 4: Representative photomicrographs of Positive AR immunostaining, Intensity of staining A) strong, B) moderate, C) weak, (400X)

We performed a cross-section analytical study at the pathology department of the Faculty of Medicine at Suez Canal University. Thirty-five prostatic specimens with prostate cancer were analyzed for immunohistochemical expression AR. Our study revealed that the percentage of AR-positive specimens is higher than negative ones, where 85.7% (n=30) of specimens were AR-positive and 14.3% (n=5) were AR-negative. Of the 30 AR-positive specimens, 7 specimens (23.33%) showed strong staining, 16 specimens (53.33%) showed moderate staining and 7 specimens (23.33%) showed weak staining. These results match with those of Hashmi et al.[19] who reported a significantly higher percentage of AR-positive cells in prostate cancer than negative ones (with a percentage of positive cells of 56.2%) and those of Qiu et al.[7] who reported Mean percentage of AR-positive epithelial cells was significantly higher in cancer tissues than that in normal prostate tissues (mean ± SD, 90.0%± 9.3%vs. 85.3%± 9.7%, P < 0.001). Similar results were obtained by Putriyuni and Oktora[20] who found low AR expression in 48,21% (27 cases) while high AR expression was seen in 51,79% (29 cases), Henshall et al.[21] who found overexpression of AR (≥70% positive nuclei) in the malignant epithelium and loss of AR immunoreactivity in the adjacent periepithelial stroma (≤30%) and Osman et al.[16] who found that mean value of AR expression was significantly higher in prostatic carcinomas than in benign hyperplasia (P = 0.001). That didn't agree with Segawa et al.[22] who found that the expression of AR in the tumor cells (52.2 ± 27.1%) was significantly lower than that in the non-tumorous tissue (68.3 ± 8.3%; P < 0.001). In our study, there was a statistically significant association between high AR expression and higher Total Gleason score (p value=0.008) with AR-positive specimens (with a mean of 7.67) having higher total Gleason score than AR negative ones (with a mean of 6.40) & statistically significant difference between different grade groups regarding AR expression (P value =0.001). These results are in keeping with Putriyuni and Oktora[20] who reported a Significant correlation of AR expression with the Gleason score (p=0.018) . It indicated that prostate cancer with a high-grade Gleason score had 5,098 times high AR expression rather than low AR expression and Hashmi et al.[19] found a Significant association of AR expression with total Gleason score, WHO grade, and percentage of tissue involvement (tumor quantification) which are among the most important markers of tumor progression. Similar results were obtained by Inoue et al.[23], Li et al.[24], and Henshall et al.[21] who found that high AR expression is associated with a high Gleason score with a statistically significant relation. Therefore, we suggest that AR expression should be performed in patients with prostatic adenocarcinoma for prognostic stratification of the patients. However, our results disagreed with the work done by Lekshmy and Prema [15], Segawa et al.[22] and Osman et al.[16] who reported a significant negative correlation between Androgen Receptor expression and Gleason score. Also Filipovski et al.[14] showed that there was no significant statistical difference in the average values of AR expression and different grade groups (p = 0.9). This variation in study results may be due to heterogeneous

expression of AR in carcinoma prostate, difference in the antibodies used to detect AR receptor in various studies and differences in the quantitation of AR immune reactivity in different studies. Because of the difference in results of various studies and the heterogeneous expression of AR in carcinoma prostate, we need to find a standard AR immunostaining counting system that is reliable and reproducible before AR immunostaining can become a valuable molecular marker of prostatic carcinoma. Li et al.[24] reported high expression of AR was predictive of a higher probability of recurrence (P = 0.0046) and high levels of AR expression also correlated with a high Ki-67 index showing high correlation (P = 0.0000) thus high levels of AR are associated with increased proliferation. This confirms the role of AR in tumor growth and progression in PCa. An interesting finding documented by Shokeir et al.[2] that stromal AR was significantly related to the percentage of PCa in the specimens (P=0.003), tumor grade (P=0.001), perineural invasion (P=0.041), and cancer stage (P=0.001) suggesting that AR expression in prostatic cancer stroma may have protective value against cancer progression, they identify the need to further investigate the mechanistic basis of loss of AR expression in the malignant stroma and its potential role in deregulation of prostate epithelial cell proliferation. In the current study, there was no statistically significant correlation between peineural invasion and AR expression (P value =0.271). Similar results were obtained by Putriyuni et al.[20] who found that there is no significant correlation between AR expression & perineural invasion (p value=0.830) and Hashmi et al.[19] who found a statistically insignificant association of AR expression with other variables including perineural invasion, lymphovascular invasion, extra-prostatic extension, and seminal vesicle invasion. In this study, age was not significantly associated with AR immunohistochemical expression in prostatic cancer. That was concordant with Lekshmy and Prema,[15] and Husain et al.[17] results. In addition, no previous studies were displaying significant association between AR expression and the age of the patients; indicating that AR expression may not be dependent on the age of patients.

Conclusion

This study focused on evaluating the relationship of Androgen Receptor expression with various prognostic factors associated with prostatic carcinoma. AR was upregulated in prostate cancer; mainly in cancers with a worse prognosis which have higher Gleason Score and higher-grade groups. So, we suggest a role played by AR in the progression of prostate cancer that can be used as a predictive tool or therapeutic target.

References

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87-108.
2. Mohamed H, Sherif MM, Ragheb AM, et al. Pcn487 Cost of Prostate Cancer and Quality of Life in Egypt-a Patient Perspective Study. Value Heal. 2019;22:S531-S532.
3. Shokeir FAA, Abd Allah MYY, El-Karef AA, et al. Stromal androgen receptor expression in prostate cancer in relation to clinico-

pathological parameters. Egypt J Pathol. 2019;39(1):79-88.
4. Abdel-Hady A, El-Hindawi A, Hammam O, et al. Expression of ERG protein and TMRPSS2-ERG fusion in prostatic carcinoma in Egyptian patients. Open Access Maced J Med Sci. 2017;5(2):147.
5. Ghei M, Pericleous S, Kumar A, et al. Finger-guided transrectal biopsy of the prostate: a modified, safer technique. Ann R Coll Surg Engl. 2005;87(5):386.
6. Greene KL, Albertsen PC, Babaian RJ, et al. Prostate specific antigen best practice statement: 2009 update. J Urol. 2013;189(1):S2-S11.
7. Qiu Y, Leuschner I, Braun PM. Androgen receptor expression in clinically localized prostate cancer: immunohistochemistry study and literature review. Asian J Androl. 2008;10(6):855-863.
8. Ding Y, Yuan H, Kong F, et al. Ectopic expression of neurotrophic peptide derived from saposin C increases proliferation and upregulates androgen receptor expression and transcriptional activity in human prostate cancer cells. Asian J Androl. 2007;9(5):601-609.
9. Altuwaijri S, Wu C, Niu Y, et al. Expression of human AR cDNA driven by its own promoter results in mild promotion, but not suppression, of growth in human prostate cancer PC-3 cells. Asian J Androl. 2007;9(2):181-188.
10. Lee D. High androgen receptor levels are predictive of decreased survival in prostate cancer. Clin Prostate Cancer. 2003;2(1):13-14.
11. Allred DC. Issues and updates: evaluating estrogen receptor-α, progesterone receptor, and HER2 in breast cancer. Mod Pathol. 2010;23:S52-S59.
12. Mukherjee T. Interpretation of ER and Her2neu hormonal receptor in breast cancer. Med Journal, Armed Forces India. 2016;72(1):99.
13. Megas G, Chrisofos M, Anastasiou I, et al. Estrogen receptor (α and β) but not androgen receptor expression is correlated with recurrence, progression, and survival in post-prostatectomy T3N0M0 locally advanced prostate cancer in an urban Greek population. Asian J Androl. 2015;17(1):98-105.
14. Filipovski V, Kubelka-Sabit K, Jasar D, et al. Androgen receptor expression in epithelial and stromal cells of prostatic carcinoma and benign prostatic hyperplasia. Open Access Maced J Med Sci. 2017;5(5):608.
15. Lekshmy KS, Prema NS. Study of Various Prognostic Factors in Prostate Cancer and its Correlation with Androgen Receptor Expression. J Evol Med Dent Sci. 2019;8 (34):2687-2694.
16. Osman WM, Abd El Atti RM, Abou Gabal HH. DJ-1 and androgen receptor immunohistochemical expression in prostatic carcinoma: a possible role in carcinogenesis. J Egypt Natl Canc Inst. 2013;25(4):223-230.
17. Husain I, Shukla S, Soni P, et al. Role of androgen receptor in prostatic neoplasia versus hyperplasia. J Cancer Res Ther. 2016;12(1):112-116.
18. Park IA, Sa HS, Chung YS, et al. Androgen receptor-positive ductal adenocarcinoma of the nasolacrimal duct: a case report. Am J Ophthalmol Case Reports. 2017;5: 33-37.
19. Hashmi AA, Mudassir G, Irfan M,et al. Prognostic significance of high androgen receptor expression in prostatic acinar adenocarcinoma. Asian Pacific J cancer Prev APJCP. 2019;20(3):893.
20. Hermien H, Cangara H, Miskad UA, et al. The role of androgen receptor expression in prostate adeno-

carcinoma. Open Access Maced J Med Sci. 2022;10(A):1263-1267.
21. Henshall SM, Quinn DI, Lee CS, et al. Altered expression of androgen receptor in the malignant epithe-
22. lium and adjacent stroma is associated with early relapse in prostate cancer. Cancer Res. 2001;61(2):423-427.
23. Segawa N, Mori I, Utsunomiya H, et al. Prognostic significance of neuroendocrine differentiation, proliferation activity and androgen receptor expression in prostate cancer. Pathol Int. 2001;51(6):452-459.
24. Inoue T, Segawa T, Shiraishi T, et al. Androgen receptor, Ki67, and p53 expression in radical prostatectomy specimens predict treatment failure in the Japanese population. Urology. 2005;66(2):332-337.
25. Li R, Wheeler T, Dai H, et al. High level of androgen receptor is associated with aggressive clinicopathologic features and decreased biochemical recurrence-free survival in prostate: cancer patients treated with radical prostatectomy. Am J Surg Pathol. 2004;28(7):928-934.