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# Association of Connexin 32 with Prostate Volume and PSA Level in Prostatic Adenocarcinoma and Adenomyomatous Hyperplasia

## Prostat Adenokarsinomu ve Adenomyomatöz Hiperplazide Prostat Hacmi ve PSA Düzeyi ile Connexin 32 İlişkisi

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**What's known on the subject? and What does the study add?**

Prostate cancer (PC) is the second most common cancer in men in the world. PC is associated with many parameters. Connexins may also be prognostic parameters in this study, we aimed to evaluate Cx32 expression in prostatic adenocarcinomas and adenomyomatous hyperplasia. In addition, age, prostate-specific antigen level, prostate volume, Gleason score (and grade group) were also evaluated.

## Abstract

**Objective:** Prostate cancer (PC) is the second most common cancer in men in the world. PC is associated with many parameters. Connexins may also be prognostic parameters in this study, we aimed to evaluate Cx32 expression in prostatic adenocarcinomas (PCa) and adenomyomatous hyperplasia (AH). In addition, age, prostate-specific antigen (PSA) level, prostate volume (PV), Gleason score (GS) (and grade group) were also evaluated.
**Materials and Methods:** This study was conducted on a total of 48 cases including 23 PCa and 25 AH.
Prostate samples were stained with Cx32 antibody by immunohistochemical method. Age, GS (and grade group), PV and PSA values were recorded. Cx32 staining intensity of the cases were evaluated statistically with these parameters.
**Results:** The age range of patients with PCa and AH was 46-83 years and 50-86 years, respectively.
The PV in PCa patients and AH patients ranged from 21 to 135 mL and from 36 to 110 mL, respectively.
The PSA value in PCa and AH patients ranged from 1 to 1122 ng/mL and from 1 to 16 ng/mL, respectively.
In cases with PCa, no statistically significant correlation was observed between GS and age and intensity of CX32 staining (p=0.523 and p=0.093, respectively). However, the mean age of Cx32-positive patients was higher than that of Cx32-negative patients (72.62 vs 67.03). The rate of Cx32-positive PCa cases was higher than that of AH cases (39.1% vs 24%).
**Conclusion:** Cx32 expression tended to increase with age. However, no significant relationship was found between PCa and AH and Cx32.
**Keywords:** Prostatic adenocarcinomas, Adenomyomatous hyperplasia, Prostate-specific antigen, Prostate volume

## Öz

**Amaç:** Prostat kanseri, dünyada erkeklerde en sık görülen ikinci kanserdir. Prostat kanseri birçok parametreyle ilişkilidir. Belki de konnexinler de prognostik parametre olabilirler. Bu çalışmada, prostat adenokarsinomu (PCa) ve adenomyomatöz hiperplazide (AH) Cx32 ekspresyonunu değerlendirmeyi amaçladık. Ayrıca yaş, prostat spesifik antijen (PSA) düzeyi, prostat hacmi (PV), Gleason skoru (GS) (ve grade grubu) da değerlendirildi.
**Gereç ve Yöntem:** Bu çalışma 23 PCa ve 25 AH dahil toplam 48 olgu üzerinde gerçekleştirildi. Prostat örnekleri immünohistokimyasal yöntemle Cx32 antikoru ile boyandı. Yaş, GS, PV ve PSA değerleri kaydedildi. Olguların Cx32 boyama şiddeti bu parametrelerle istatistiksel olarak değerlendirildi.
**Bulgular:** PCa ve AH hastalarının yaş aralığı sırasıyla 46-83 ve 50-86 idi. PCa hastalarında ve AH hastalarında PV sırasıyla 21 ila 135 mL ve 36 ila 110 mL arasında değişmekteydi. PCa ve AH hastalarındaki PSA değeri sırasıyla 1 ila 1122 ng/mL ve 1 ila 16 ng/mL arasında değişmekteydi. PCa olgularında Cx32 boyama şiddetinin GS ve yaş arasında istatistiksel olarak anlamlı bir ilişki gözlenmemiştir (sırasıyla p=0,523 ve p=0,093). Bununla birlikte, Cx32 pozitif olguların ortalama yaşı, Cx32 negatif olguların yaşından daha yüksekti (72,62 vs 67,03). Yüzde olarak, Cx32 pozitif PCa olgu sayısı AH olgu sayısından daha yüksekti (%39,1/%24).

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**Sonuç:** Cx32 ekspresyonu yaşla birlikte artma eğilimindedir. Bununla birlikte, PCa ile AH ve Cx32 arasında anlamlı bir ilişki bulunamadı.
**Anahtar Kelimeler:** Prostat adenokarsinomları, Adenomyomatöz hiperplazi, PSA, Prostat hacmi.

## Introduction

Prostate cancer (PC) is the second most commonly occurring cancer and the fifth leading cause of death in men worldwide (1,2).

There is great variability in the biological behavior and metastatic potential of prostatic adenocarcinomas (PCa). The effectiveness of treatment depends on age, medical parameters, stage, grade and available medical facilities (1,2,3).

Prostate-specific antigen (PSA) testing and digital rectal examination are recommended for the detection of PCa (4,5). Serum PSA level is the most commonly used marker to detect this cancer in the general population (4,5). However, PSA is prostate-specific but not disease-specific. As a result, it is not sufficient alone in PCa screening (4,5).

Practical and feasible PC staging and classification will guide treatment options and help determine the prognosis of PC (6,7,8). PSA level, pathological tumor [the extent of primary tumor (pT)] level and Gleason score (GS) are very important prognostic factors. However, these parameters may not be sufficient for diagnosis and follow-up (6,7,8,9,10).

Gap junction (GJ) channel proteins include pannexines, innexins and connexins (Cx) (11,12,13). The classification of Cx was based on two systems: the first is the molecular weight in the cDNA sequence. So, Cx32 and Cx43 correspond to the molecular weights of 32 kDa and 43 kDa (14). The second is based on the sequence similarity and length of the cytoplasmic domain of the Cx (classifying them into $\alpha$, $\beta$, and $\gamma$ subgroups) (15). GJs play an important role in cell-to-cell transition controls. GJs provide intercellular communication through water-soluble molecules. The major cellular function is the control of homeostasis. They play an important role in several functions such as reproduction, differentiation and migration. In addition, they also play an important role in homeostasis, tumor suppression and other cellular functions. Homeostatic imbalance may lead to a variety of diseases including malignant tumors (11,12,13).

Cx32 expression is observed in acinar cells of exocrine glands such as prostate and pancreas (16).

The aim of this study was to evaluate the relationship of Cx32 with prostate volume (PV), PSA, GS and grade group in PCa and adenomyomatous hyperplasia (AH).

## Materials and Methods

This study is a retrospective study. The study protocol was reviewed and approved by the Ordu University Ethics Committee with the approval number 2016/43. This study was conducted on a total of 48 paraffin-embedded prostate samples, which were histopathologically diagnosed at the Department of Pathology between 2014 and 2015. The samples consisted of 23 PCa and 25 AH. Pathological materials of cases diagnosed with PCa were radical prostatectomy and tru-cut biopsy specimens. The GS and grade group distribution of the samples were as follows; 2 cases: GS 4 (grade group 1), 11 cases: GS 6 (grade group 1), 2 cases: GS 3+4 (grade group 2), 1 case: GS 4+3 (grade group 3), 4 cases: GS 8 (grade group 4), and 3 cases: GS 9 (grade group 5). Age, GS, PV and PSA values of the patients were evaluated. These parameters were compared with Cx32 staining. Paraffin-embedded prostate samples were cut at 3 µm thickness. These sections were immunostained with Cx32 antibody.

### Immunohistochemistry

The sections were kept at 60 °C for 1 hour, then xylol and alcohol steps were applied. The sections were incubated in a 3% hydrogen peroxide solution for 10 minutes, then, washed for 5 minutes in distilled water. Antigen was retrieved through retrieval step. Immunohistochemical staining was performed using the avidin-biotin complex technique. The antigen was washed in phosphate buffered saline after the retrieval protocol. Primary antibody Cx32 (dilution ratio 1:200) was applied. The sections were rinsed in 3-amino-9-ethylcarbazole and chromogen substrate (10 minutes), washed with water, stained with hematoxylin (3 minutes) and covered with mounting medium, respectively.

The stained sections were examined with a Nikon Eclipse Niu microscope and photos were taken.

Cx32 scoring was evaluated semi-quantitatively. The evaluation was made as follows; no staining (score 0), weak (score 1), strong (score 2) (Figure 1,2) (17). In addition, gland and stromal cells were evaluated.

In order to obtain statistically significant results, the cases showing weak and strong staining were evaluated as the group showing positive staining, and in this way, two groups were formed as negative and positive groups.

### Statistical Analysis

A chi-square test was used to investigate the relationship between Cx32 staining results (negative, positive) and biopsy diagnoses (PCa, AH) after pathological examination of the samples taken from patients in the study.

In addition, the independent samples t-test, Fisher-Freeman-Halton exact test and the Mann-Whitney U test ($p<0.05$) were applied to reveal the relationship between Cx32 staining results and biopsy diagnosis.

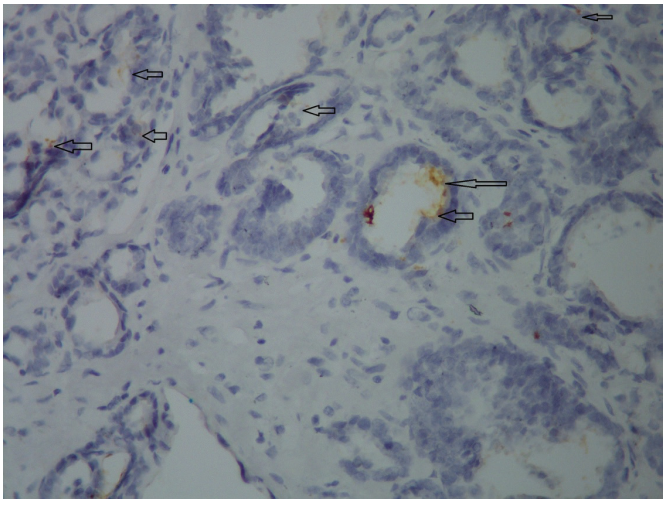

**Figure 1.** Cx32 expression (strong degree) was observed in malignant glands. The staining showed apical, membranous (Cx32 x400)

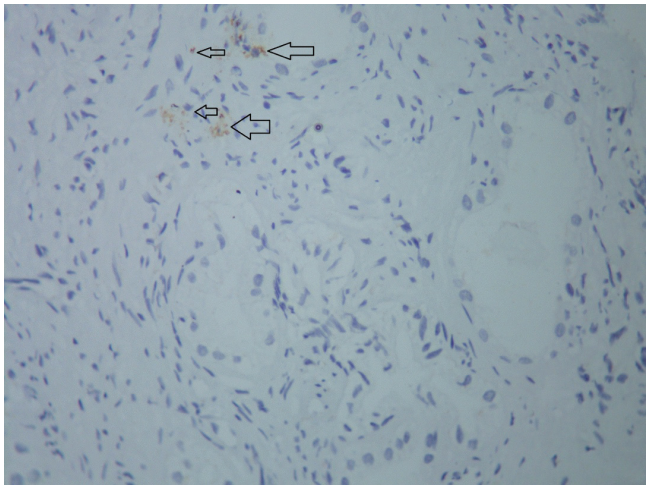

**Figure 2.** Cx32 expression (middle degree) was observed in adenomyomatous hyperplasia. The staining showed apical, membranous (Cx32 x 200)

Mann-Whitney U test was used to evaluate patient age, PSA and PV. All statistical calculations were made using the SPSS v.19.0 program.

## Results

Descriptive statistics including age, PSA and PV measurements in 48 samples (PCa and AH) are shown in Table 1,2. The age of the PCa and AH patients ranged from 46 to 83 years and 50 to 86 years, respectively. The age distribution of the PCa patients is as follows: 6 cases were under 65 years of age and 16 cases were over 65 years old. The PV of the PCa patients and AH patients ranged from 21 to 135 mL (determined via ultrasonographic evaluation as follows: width x height x length x 0.52) and from 36 to 110 mL, respectively. The PSA value in PCa and AH patients ranged from 1 to 1122 ng/mL and from 1 to 16 ng/mL, respectively. The mean age of the PCa and AH patients was 70.3±1061 and 68.12±10.08 years, respectively. The mean PSA value was 112.00 (min=1, max=1122 ng/mL) and 5.98±4.14, respectively. The mean PV was 60.78±31.81 and 59.00±21.73, respectively. The relationship of age with PSA and PV was evaluated in AH and PCa groups and no significant correlation was found (p=0.468, p=0.197 and p=0.820, respectively). No significant relationship of Cx32 expression with PCa and AH was found (p=0.656, Fisher-Freeman-Halton exact test) (Table 3).

No significant relationship was found between GS and Cx32 expression in PCa group (r= -0.140 and p=0.523, Spearman's correlation coefficient).

Cx32 expression was evaluated in all patients with PCa. The mean age of the Cx32-positive and -negative patients was 72.62 and 67.03 years, respectively. The mean age of Cx32-positive

**Table 2. Comparison of AH and PCa groups with age, PSA and prostate volume**

| | n | Age | PSA** | PV* |
|---|---|---|---|---|
| | | Mean | | |
| PCa groups | 23 | 70.30 | 112.00 | 60.78 |
| AH groups | 25 | 68.12 | 5.98 | 59.00 |
| p | | 0.468 | 0.197 | 0.820 |

*: Independent samples t-test; **: Mann-Whitney U test, PV: Prostate volume, PSA: Prostate-specific antigen, PCa: Prostatic adenocarcinomas, AH: Adenomyomatous hyperplasia

**Table 1. Values for age, PSA and prostate volume measurements**

| | n | Mean | Min | Max | Percentiles | | |
|---|---|---|---|---|---|---|---|
| | | | | | 25th percentile | Median | 75th percentile |
| **Age** | 48 | 69.1667 | 46.00 | 86.00 | 60.2500 | 68.0000 | 79.7500 |
| **PSA** | 48 | 56.7796 | 0.69 | 1122.0 | 3.2800 | 5.3800 | 10.1100 |
| **PV** | 48 | 59.8542 | 21.00 | 135.00 | 44.2500 | 52.0000 | 67.0000 |

PV: Prostate volume, PSA: Prostate-specific antigen

patients was higher than -negative patients (72.62 vs. 67.03). The rate of Cx32-positive PCa cases was higher than that ofAH cases (39.1% vs. 24%). There was no statistically significant relationship between Cx32 expression and age (p=0.093) (Table 4).

**Table 3. The distribution of Cx32 results according to AH and PCa groups**

| Count | | AH groups | | PCa groups | |
|---|---|---|---|---|---|
| | | % | Count | % | |
| Cx32 | negative | 19 | 76.0 | 14 | 60.9 |
| | mild | 5 | 20.0 | 8 | 34.8 |
| | strong | 1 | 4.0 | 1 | 4.3 |
| Total | | 25 | - | 23 | p=0.656 |

PCa: Prostatic adenocarcinomas, AH: Adenomyomatous hyperplasia

**Table 4. The distribution of Cx32 results according to age**

| Cx32 | | n | Mean | Std. Deviation | |
|---|---|---|---|---|---|
| Age | negative | 33 | 67.03 | 9.95 | p=0.093 |
| | positive | 15 | 72.62 | 9.87 | |

## Discussion

There are many studies on PSA levels, rate of positive biopsies, lymph node metastasis, positivity of surgical margins, extracapsular enlargement and seminal vesicle invasion, GS, and biochemical recurrence. These studies have tried to reveal the risk of biochemical recurrence, especially after radical prostatectomy (18,19,20,21,22,23). GJs or Cx are space junctions composed of protein subunits. GJs have an important role in cellular communication, growth, differentiation and carcinogenesis. GJ dysfunction has been reported to be associated with various cancers and diseases (21,22,23,24).

Jee et al. (24) reported that Cx32 was expressed at intercellular contact points in normal cells and showed punctate intercellular and intracyloplasmic staining in cancer cells. It was also found that the frequency of altered Cx32 expression in adenocarcinoma was significantly higher compared to that in normal mouse stomach. The expression pattern of Cx32 in mouse gastric cancer model was similar to that in human. Cx32 was mainly expressed in the cytoplasm of epithelial cells in the mucus metaplasia of mouse stomach. There was also an inverse correlation between Cx32 expression and cell proliferation in mouse tumors. In terms of mRNA levels, there was no difference between normal and cancerous tissues (24).

Fujimoto et al. (25) found that Cx32 suppressed the Src-Stat3-vascular epithelial growth factor (VEGF) signaling and thus had a tumor suppressor effect against a metastatic renal cell carcinoma (RCC) cell line (Caki-1 cell) *in vivo*. They suggested that Cx32 was a promising molecular target for potential new cancer therapy due to the effects on angiogenesis. They reported a close relationship between Cx32 expression level, Src/Stat3 signaling activation and VEGF production in invasive and metastatic RCC tissues (25).

Xu et al. (26) assessed the association between Cx43 expression and clinicopathologic features of PCa and biochemical recurrence after radical prostatectomy. They found that Cx43 protein significantly decreased or disappeared in PCa compared to AH tissues. It was reported that reduced Cx43 expression was associated with advanced clinicopathological features (26).

Saladino et al. (27) evaluated Cx43, Cx32 and Cx26 expressions in non-tumorigenic and tumorigenic human prostate epithelial cells. In their study, there was an inverse relationship between the expression levels of Cx43 and Cx32. They reported that Cx43 was largely expressed in non-tumorigenic cells while Cx32 was predominantly expressed in tumorigenic cells (27).

In their study investigating Cx26, Cx32 and Cx43 expressions in paraffin samples obtained from patients with PCa and AH, Hu et al. (28) found positive expressions of Cx32 in 78.3% of AH and 61.3% of PCa samples (p>0.05). In this study, positive Cx32 staining was observed in stromal cells and glandular epithelium (Figure 1,2).

Staining was seen in cytoplasmic and cytoplasmic membrane. The degree of staining was mild and did not differ significantly in terms of benign and malign differentiation. In this study, Cx32 staining was positive in 24% of AH patients and 40% of PCa patients. It was observed that the Cx32 staining rate was higher in the PCa group compared to the AH group. However, there was no statistically significant relationship between Cx32 expression and age (p=0.093). In a more recent study, it was shown that in men with benign prostatic hyperplasia (n=1859), the PV increased from 27.7 mL in the 40-49 age group to 52.3 mL in the 70-80 age group (29).

In this study, the average PV was 59 mL and the average age was 68.12 years in AH group. When compared with the literature, the reason for the high PV may be related to the older patients in this study.

Stephan et al. (30) reported that the mean PV in BPH patients was larger than in PCa group and there was a positive correlation between PV and total PSA. In this study, there was no significant difference in PV between PCa and AH groups. It was noticed that there was no significant correlation between PSA and PV. Cx32 expression exhibited no correlation with PV and PSA level in the PCa and AH groups. Serum PSA level may increase for many reasons. For example, the PSA level may increase in hyperplastic growth of prostate tissues, prostate manipulation, urinary retention, sexual activity, inflammation and cancer

(31). In this study, PSA values were higher in patients with PCa than in those with AH (112; 5.98 respectively). In this study, it was noticed that PV and PSA did not increase at the same rate. Patients with PCa are mostly over 65 years of age and PCa is rare in men younger than 50 years (32). In this study, 6 cases were under 65 years old and the others were over 65 years of age. While the prevalence of PCa in young men (aged <50 years) was 1% in the 1970s, recenty, it has increased to 20-30% (32). In this study, 1 patient with PCa was 46 years old and 6 were under 65 years old and the others were over 65 years of age.

The average age of the Cx32-negative patients was 66.06 years. The mean age of AH and PCa patients was 68.12 and 70.3 years, respectively. Cx32-negative cases were younger than Cx32-positive cases. However, loss of Cx32 expression was observed in both AH and PCa patients and no statistically significant difference was detected.

In this study, there was no correlation between Cx32, diagnosis (PCa and AH) and PSA.

## Conclusion

Cx32 expression increases with age. No significant correlation was found between Cx32 expression and GS, PV, PSA, PCa and AH. Further large-scale studies are warranted.

### Ethics

**Ethics Committee Approval:** The study protocol was reviewed and approved by the Ordu University Ethics Committee with the approval number 2016/43.

**Informed Consent:** Retrospective study.

**Peer-review:** Externally peer-reviewed.

### Authorship Contributions

Concept: H.E., Design: H.E., Data Collection or Processing: H.E., Analysis or Interpretation: S.Ç., Literature Search: H.E., Writing: H.E.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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