

Vitamin D receptor polymorphism and prostate cancer prognosis

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Abstract

Background: Prostatic epithelial cells synthesize the active form of vitamin D (1,25-dihydroxyvitamin D₃), which participates in regulating prostate growth. Calcitriol, a synthetic form of vitamin D₃, exhibits antiproliferative and prodifferentiation activities in prostate cancer. The function of 1,25-dihydroxyvitamin D₃ is mediated by its binding to vitamin D receptor (VDR). VDR forms a heterodimer, typically with retinoid X receptor, to regulate vitamin D target genes. We evaluated the relationship between VDR polymorphism and clinical characteristics associated with prostate cancer risk and prognosis among Egyptian men.

Materials and methods: This case-control study included 2 groups of patients: group A, a control group of 50 subjects with benign prostate hyperplasia, and group B, 50 subjects newly diagnosed with prostate cancer. All participants performed complete blood count, liver and kidney function tests, prostate specific antigen measurement, histopathological analysis and immunohistochemistry for Dickkopf Homolog 3. Restriction fragment length polymorphism-polymerase chain reaction as performed to detect VDR polymorphism.

Results: Patients with prostate cancer and controls showed a significantly different CA genotype frequency ($p = 0.007$). Furthermore, prostate-specific antigen levels were significantly different in different genotypes in patients with prostate cancer ($p < 0.001$). Finally, T stage and the VDR *Apal C/A* polymorphism were significantly associated ($p < 0.041$).

Conclusion: The VDR *Apal C/A* polymorphism may be a diagnostic and prognostic marker for prostate cancer in Egyptian men.

Keywords: Prostate cancer; Prostate-specific antigen; Vitamin D receptor polymorphism

1. Introduction

Prostate cancer is the most commonly diagnosed cancer and the second most common cause of death among men.^[1] Its incidence is highest among Africans, followed by Whites and Mongolians,^[2] which may be explained by differences in social, environmental, and genetic factors. In Egypt, prostate cancer accounts for approximately 4.27% of all cancers based on the National Population-Based Registry Program of Egypt 2008–2011. This number is expected to increase because the population and average life expectancy are increasing in Egypt.^[3]

However, the etiology of prostate cancer remains unclear. Risk factors for prostate cancer include aging, diet, obesity, lifestyle, ethnicity, smoking, infections, prostate gland inflammation, and vasectomy.^[4] Exposure to certain chemicals and genetic predisposition may also be involved. In addition, oncogene activation and subsequent tumor development may be caused by structural genomic

rearrangements.^[5] Hence, genetic variations in prostate cancer-related genes, including the *vitamin D receptor* gene (VDR), may be important in determining disease susceptibility.^[6]

Vitamin D is involved in many functions other than bone metabolism, including the reduction of inflammation and modulation of cellular processes such as cell growth, neuromuscular and immune functions, and glucose metabolism.^[7] Vitamin D also modulates numerous genes encoding proteins that regulate cell proliferation, differentiation, and apoptosis. The VDR is present in many tissues and converts 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D in some of these tissues.^[8]

Measurement of the prostate-specific antigen (PSA) level is a standard test used to diagnose prostate cancer. The Food and Drug Administration approved this test in 1986 for evaluating disease progression.^[9] However, the specificity of PSA for diagnosing prostate cancer is very low,^[10] and thus, more specific molecular markers are required to enable early diagnosis and treatment, which decrease mortality in patients with prostate cancer.

Prostate cell division is influenced by the steroid hormones testosterone and vitamin D, the actions of which are mediated by the androgen receptor (AR) and VDR, respectively.^[11] Prostate epithelial cells express multiple members of the nuclear receptor superfamily that regulate cell proliferation and differentiation in the prostate gland. VDR is a nuclear hormone receptor that regulates gene transcription. Although this regulation has been shown to be disturbed in prostate cancer,^[12] the role of the VDR in prostate

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cancer remains controversial. In the Taiwanese (Asian) population, the frequency of any genotype and prostate cancer showed no association.^[13] In contrast, other studies suggested that *Apal* polymorphism confers susceptibility to sporadic disease.^[14,15]

Based on the controversial role of VDR in prostate cancer, studies are needed to determine whether VDR polymorphisms are useful as diagnostic and prognostic markers among Egyptian patients.

2. Materials and methods

2.1. Patients

This prospective study was conducted in the Department of Clinical Oncology and Nuclear Medicine, Zagazig University Hospitals, and Medical Biochemistry and Molecular Biology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt, in collaboration with the Zoology Department, Faculty of Science, Zagazig University.

This was a case-control study performed to evaluate 2 groups. Group A (control group) included 50 patients with benign prostate hyperplasia. Group B included 50 patients newly diagnosed with prostate cancer as confirmed by histopathological analysis. Participants provided written informed consent after the procedures and possible hazards were explained. The study was approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University.

Inclusion criteria for the patients were as follows: age of between 50 and 85 years, histologically confirmed adenocarcinoma of the prostate without evidence of neuroendocrine or small cell differentiation, Gleason score ≥ 7 , any stage including low-volume metastatic disease, Eastern Cooperative Oncology Group performance status (ECOG PS) between 0 and 2, eligible for hormonal treatment, and adequate organ function. The volume of the disease as a potential predictor was introduced by the chemohormonal therapy versus androgen ablation randomized trial for extensive disease in prostate cancer based on imaging with a bone scan and computed tomographic scanning. High-volume metastasis was defined as ≥ 4 bone metastases, including ≥ 1 outside the vertebral column or pelvis and/or visceral metastasis.^[14]

Exclusion criteria included an age of <50 or >85 years, unavailable histology, ECOG PS >2 , severe concurrent illness, or comorbid disease that would make the subject unsuitable for enrollment.

All eligible patients with prostate cancer were evaluated, treated, and followed-up at the Clinical Oncology and Nuclear Medicine Department. Eligible patients were enrolled from January 2018 to April 2019. Patient follow-up ended in October 2021.

All patients provided full histories and underwent physical examination and complete blood count (CBC), liver and kidney function tests, PSA measurement, histopathological analysis to confirm prostate cancer, and immunohistochemistry (IHC) for Dickkopf homolog 3 (DKK3).

The patients underwent hormonal treatment. Biochemical relapse was defined as a PSA increase of 2 ng/mL above the nadir (Phoenix Criteria, 2005).^[16] According to the D'Amico risk stratification system, patients were classified into 3 groups to predict posttreatment biochemical failure. Low risk was defined as 1992 AJCC T1/T2a, PSA level ≤ 10 ng/mL, and Gleason score of ≤ 6 . Intermediate risk was defined as 1992 AJCC T2b and/or PSA of 10 to 20 ng/mL, and/or Gleason score of 7. High-risk disease was classified as any one of the following high-risk features: 1992 AJCC $\geq T2c$, PSA >20 ng/mL, or Gleason score of 8 to 10.

2.2. Immunohistochemistry

Immunohistochemical staining was performed using the polymer Envision Detection System and Dako EnVision kit (Dako, Glostrup, Denmark). Tissue sections (3–5 μm) were deparaffinized in xylene and rehydrated in graded alcohol. The slides were incubated for 10 minutes in 3% hydrogen peroxide to block endogenous peroxidase activity, followed by treatment with Dako Target Antigen Retrieval Solution (pH 6.0). The slides were incubated with an anti-DKK3 antibody (ab187532; Abcam, Cambridge, UK). The reaction was visualized by incubating the sections with diaminobenzidine for 15 minutes. The sections were stained with Mayer hematoxylin. Immunohistochemical scoring was performed by a pathologist using a graded scale from 0 to 3+, where 0 to 2 represented weak staining, 2+ to 3 represented moderate staining, and 3+ represented strong staining.

2.3. Biochemical and molecular procedures

Random blood glucose, total bilirubin, direct bilirubin, alanine transaminase, aspartate transaminase, albumin, urea, and creatinine

Table 1

Difference in clinicopathological features between the studied groups.

Variables	Mean \pm SD		t test	p
	Controls (n = 50)	Prostate cancer (n = 50)		
Age, yr	68.9 \pm 6.78	62.9 \pm 6.33	0.27	0.76
Hemoglobin, g/dL	13.2 \pm 1.45	12.1 \pm 1.69	3.56	0.001*
Red blood cells, cells $\times 10^6/\mu\text{L}$	4.86 \pm 0.46	4.61 \pm 0.41	2.92	0.005*
White blood cells, cells $\times 10^3/\mu\text{L}$	7.04 \pm 2.47	7.87 \pm 2.74	1.63†	0.08
Platelets, cells $\times 10^3/\mu\text{L}$	232.5 \pm 58.3	233.6 \pm 75.1	0.08	0.94
Prothrombin time, s	13.97 \pm 0.66	13.9 \pm 0.64	0.85	0.399
RBG, mg/dL	121.4 \pm 32.1	114.5 \pm 30.9	1.1	0.29
Total bilirubin, mg/dL	0.82 \pm 0.12	0.87 \pm 0.12	2.31	0.02*
Direct bilirubin, mg/dL	0.16 \pm 0.04	0.18 \pm 0.04	2.63	0.01*
Alanine transaminase, U/L	18.6 \pm 8.82	23.4 \pm 13.1	1.13†	0.08
Aspartate transaminase, U/L	21.2 \pm 9.27	24.6 \pm 12.6	1.37†	0.17
Albumin, g/dL	4.23 \pm 0.44	4.31 \pm 0.41	0.91	0.37
Creatinine, mg/dL	0.96 \pm 0.19	1.01 \pm 0.26	1.11	0.26
Urea, mg/dL	26.3 \pm 7.14	27.5 \pm 9.04	0.42	0.68
Prostate-specific antigen, ng/mL	1.86 \pm 1.18	85.4 \pm 34.2	8.62	$<0.001^\ddagger$

RBG = random blood glucose; SD = standard deviation.

* $p < 0.05$ (significant).

†Mann-Whitney test.

‡ $p < 0.001$ (highly significant).

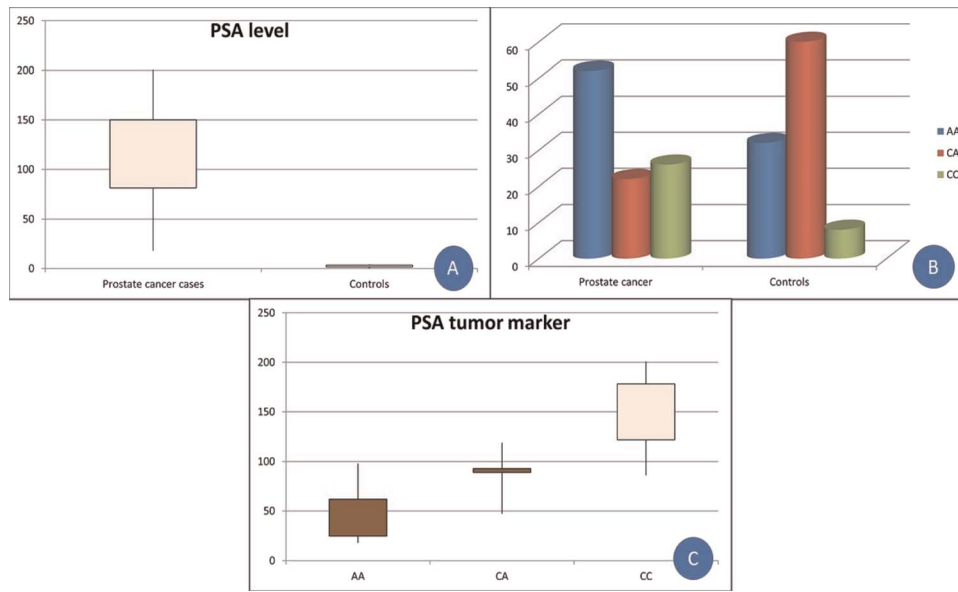


Figure 1. (A) Box-plot analysis of PSA levels among patients with prostate cancer and controls. (B) Genotypes distribution of the *VDR* gene among patients with prostate cancer and controls. (C) Box-plot analysis of PSA levels in genotypes of *VDR* among prostate cancer cases. PSA = prostate-specific antigen; *VDR* = vitamin D receptor.

were measured using a semiautomated chemistry analyzer (Sunostik Medical Technology, Changchun, China) using commercially available kits (Spinreact, Girona, Spain). A CBC was determined using a BeneSphera (Avantor, Radnor, PA).

DNA was extracted from EDTA blood samples using a commercially available G-spin Total Genomic DNA Extraction Kit (iNtron Biotechnology, Seongnam, Korea). Polymerase chain reaction (PCR) amplification was performed using a thermal cycler (PerkinElmer, Waltham, MA). The 25- μ L PCR sample mixtures contained 100-ng genomic DNA, 1.0 μ M of each primer (SBS, Beijing, China), and 12.5 μ L of 2X TOP simple PreMIX n-Taq master mix (Enzymomics, Daejeon, Korea). The primer sequences were as follows: F, 5'-CAGAGCATGGACAGGGAGCAA-3' and R, 5'-GCAACTCCTCATGGCTGAGGTCTC-3'. The PCR products were digested with the restriction enzyme *ApaI* (Enzymomics), and the fragments were separated using 2% agarose gel electrophoresis with a 100-bp marker. The gels were stained with ethidium bromide and visualized using a UV transilluminator.

2.4. Statistical analysis

Continuous variables are expressed as the mean \pm standard deviation and median (range), and categorical variables are expressed as percentages. Variables presented as percentages were compared using the Pearson χ^2 test or Fisher exact test, when appropriate. Disease-free survival

(DFS) was calculated as the time from the date of local treatment to the date of relapse or censored at the last follow-up visit. Overall survival (OS) was calculated as the time between the date of diagnosis and date of death or final follow-up visit. DFS and OS were stratified based on the DKK3 IHC results in malignant cells and *VDR ApaI C/A* polymorphism. These time-to-event distributions were estimated using Kaplan-Meier plots and compared using 2-sided exact log-rank tests. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS version 22.0 software for Windows (SPSS, Inc., Chicago, IL) and MedCalc Statistical Software version 18.9.1 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2018).

3. Results

3.1. Clinical and biochemical characteristics of study subjects

The 2 groups did not significantly differ in terms of age ($p = 0.76$). There was a significant decrease in hemoglobin and red blood cell counts in patients with prostate cancer compared with the controls ($p = 0.001$ and 0.005 , respectively), whereas other CBC parameters did not significantly differ between groups ($p > 0.05$). Total and direct bilirubin levels were significantly increased among prostate cancer cases ($p = 0.02$ and 0.01 , respectively), whereas other liver and kidney function tests showed no significant difference between the 2 groups ($p > 0.05$).

Table 2
Different genotypes and allele distributions of *vitamin D receptor* gene among the studied groups.

Genotype	Group		χ^2	<i>p</i>	Odds ratio (95% confidence interval)
	Prostate cancer (n = 50)	Controls (n = 50)			
AA	26 (52%)	16 (32%)	-	-	1
CA	11 (22%)	30 (60%)	3.14	0.007*	0.23 (0.09–0.57)
CC	13 (26%)	4 (8%)	1.06	0.29	2 (0.55–7.2)
A allele	63 (63%)	62 (62%)	0.03	0.97	0.96 (0.54–1.7)
C allele	37 (37%)	38 (38%)			

* $p < 0.05$ (significant).

A highly significant increase in pretreatment PSA levels was observed among prostate cancer cases ($p < 0.001$; Table 1, Fig. 1A).

3.2. Vitamin D receptor gene polymorphism

CA genotype differed significantly between patients with prostate cancer and controls ($p = 0.007$). However, the distribution of A and C alleles did not differ between groups ($p = 0.97$) (Table 2, Fig. 1B). In addition, PSA levels were significantly different in the genotypes of group B ($p < 0.001$; Table 3, Fig. 1C). However, the VDR polymorphism across the 3 D'Amico risk groups did not significantly differ ($p = 0.68$; Table 3).

3.3. Histopathological results

The benign prostatic gland showed corpora amylacea. Benign glands had undulating luminal contours with tufts and papillary

infolding. Tall secretory epithelial cells had a pale clear cytoplasm and uniform round or oval nuclei. In well-differentiated prostate cancer sections, the malignant glands exhibited nuclear enlargement and hyperchromasia, prominent nucleoli, intraluminal blue mucin, and the absence of a basal cell layer. In moderately differentiated prostate cancer, the malignant glands were arranged back-to-back with little intervening stroma and cribriform patterns. Poorly differentiated prostate cancer showed solid groups of malignant epithelial cells with nuclear enlargement and abnormal mitotic figures (Fig. 2).

3.4. Relation between clinicopathological features and DKK3 IHC in malignant cells

High and low expression of DKK3 was detected in 30% and 70% of patients with prostate cancer, respectively (Fig. 3). The pretreatment PSA and DKK3 IHC showed a significant association in malignant

Table 3
Relation between clinicopathological features and DKK3 IHC in malignant cells/VDR Apal genotypes in patients with prostate cancer (n = 50).

Characteristics	DKK3 IHC in malignant cells				VDR Apal C/A polymorphism			
	Prostate cancer patients (n = 50), n (%)	Low (n = 35), n (%)	High (n = 15), n (%)	p*	CC (n = 13), n (%)	AA (n = 26), n (%)	CA (n = 11), n (%)	p*
Age group, yr								
<60	24 (48)	16 (66.7)	8 (33.3)	0.621	5 (20.8)	11 (45.8)	8 (33.3)	0.173
≥60	26 (52)	19 (73.1)	7 (26.9)		8 (30.8)	15 (57.7)	3 (11.5)	
Pretreatment prostate-specific antigen, ng/mL								
<10	16 (32)	13 (81.2)	3 (18.8)	0.011 [†]	125.4 ± 27.8	64.9 ± 20.55	86.4 ± 24.4	<0.001 [†]
10–20	13 (26)	12 (92.3)	1 (7.7)					
>20	21 (42)	10 (47.6)	11 (52.4)					
ECOG PS								
ECOG 0	14 (28)	11 (78.6)	3 (21.4)	0.444	3 (21.4)	10 (71.4)	1 (7.1)	0.093
ECOG 1	19 (38)	14 (73.7)	5 (26.3)		8 (42.1)	7 (36.8)	4 (21.1)	
ECOG 2	17 (34)	10 (58.8)	7 (41.2)		2 (11.8)	9 (52.9)	6 (35.3)	
Gleason score								
Gleason 7	6 (12)	3 (50)	3 (50)	0.630	1 (16.7)	4 (66.7)	1 (16.7)	0.846
Gleason 8	23 (46)	16 (69.6)	7 (30.4)		6 (26.1)	12 (52.2)	5 (21.7)	
Gleason 9	15 (30)	11 (73.3)	4 (26.7)		3 (20)	8 (53.3)	4 (26.7)	
Gleason 10	6 (12)	5 (83.3)	1 (16.7)		3 (50)	2 (33.3)	1 (16.7)	
≤7	6 (12)	3 (50)	3 (50)	0.348	1 (16.7)	4 (66.7)	1 (16.7)	0.741
>7	44 (88)	32 (72.7)	12 (27.3)		12 (27.3)	22 (50)	10 (22.7)	
Tumor								
T2	20 (40)	16 (80)	4 (20)	0.435	7 (35)	8 (40)	5 (25)	0.041 [†]
T3	20 (40)	13 (65)	7 (35)		5 (25)	14 (70)	1 (5)	
T4	10 (20)	6 (60)	4 (40)		1 (10)	4 (40)	5 (50)	
Node								
N0	22 (44)	19 (86.4)	3 (13.6)	0.025 [†]	7 (31.8)	9 (40.9)	6 (27.3)	0.380
N1	28 (56)	16 (57.1)	12 (42.9)		6 (21.4)	17 (60.7)	5 (17.9)	
Metastasis								
M0	39 (78)	28 (71.8)	11 (28.2)	0.713	11 (28.2)	21 (53.8)	7 (17.9)	0.413
M1	11 (22)	7 (63.6)	4 (36.4)		2 (18.2)	5 (45.5)	4 (36.4)	
D'Amico risk								
Low					2 (25)	4 (50)	2 (25)	0.68
Intermediate					6 (37.5)	8 (50)	2 (12.5)	
High					5 (19.2)	14 (53.8)	7 (26.9)	
DKK3 IHC in malignant cells								
Low	35 (70)				11 (31.4)	16 (45.7)	8 (22.9)	0.325
High	15 (30)				2 (13.3)	10 (66.7)	3 (20)	
VDR Apal C/A polymorphism								
CC	13 (26)	11 (84.6)	2 (15.4)	0.325				
AA	26 (52)	16 (61.5)	10 (38.5)					
CA	11 (22)	8 (72.7)	3 (27.3)					

DKK3 = Dickkopf homolog 3; ECOG PS = Eastern Cooperative Oncology Group performance status; IHC = immunohistochemistry; VDR = vitamin D receptor.

Categorical variables are expressed as number (percentage).

* χ^2 Test.

[†] $p < 0.05$ (significant).

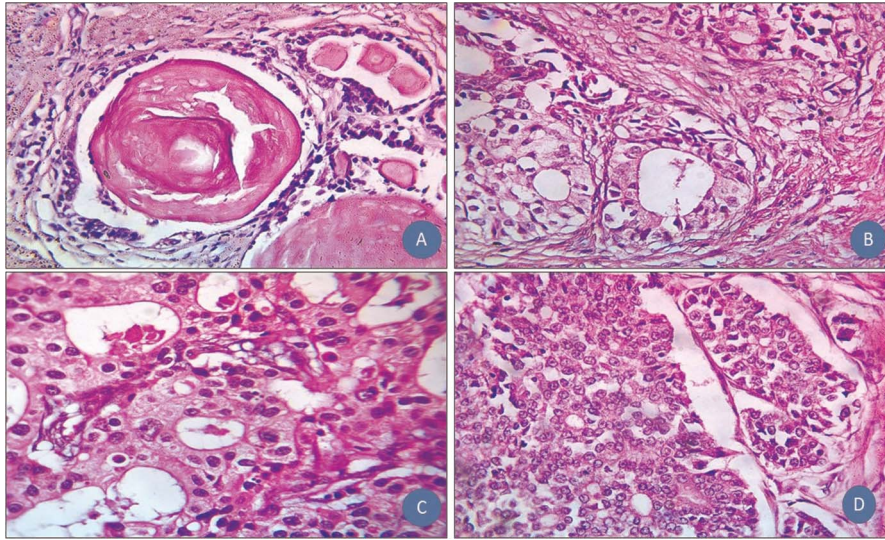


Figure 2. (A) Benign prostatic hyperplasia: contains corpora amylacea, undulating luminal contours with tufts and papillary infoldings. The tall secretory epithelial cells have a pale and clear cytoplasm and uniform round or oval nuclei (H&E, original magnification $\times 100$). (B) Well-differentiated prostate cancer: malignant glands showed nuclear enlargement and hyperchromasia, prominent nucleoli, intraluminal blue mucin, and the absence of a basal cell layer (H&E, original magnification $\times 400$). (C) Moderately differentiated prostate cancer: malignant glands are arranged back-to-back with little intervening stroma and cribriform pattern (H&E, original magnification $\times 400$). (D) Poorly differentiated prostate cancer: solid groups of malignant epithelial cells with nuclear enlargement and abnormal mitotic figures (H&E, original magnification $\times 400$). H&E = hematoxylin and eosin.

cells, where 81.2% of patients with PSA <10 ng/mL exhibited low DKK3 levels versus 47.6% of patients with PSA >20 ng/mL ($p = 0.011$). In addition, a significant association between the N stage and DKK3 IHC in malignant cells was observed; 86.4% of patients with node-negative disease had low DKK3 levels compared with 57.1% of patients with node-positive disease ($p = 0.025$; Table 3).

3.5. Relation between clinicopathological features and VDR *Apal C/A* polymorphism

T stage and VDR *Apal C/A* polymorphism showed a significant association, with 25% of patients with T2 tumors displaying a *CA* genotype versus 50% of patients with T4 tumors displaying this genotype ($p = 0.041$; Table 3).

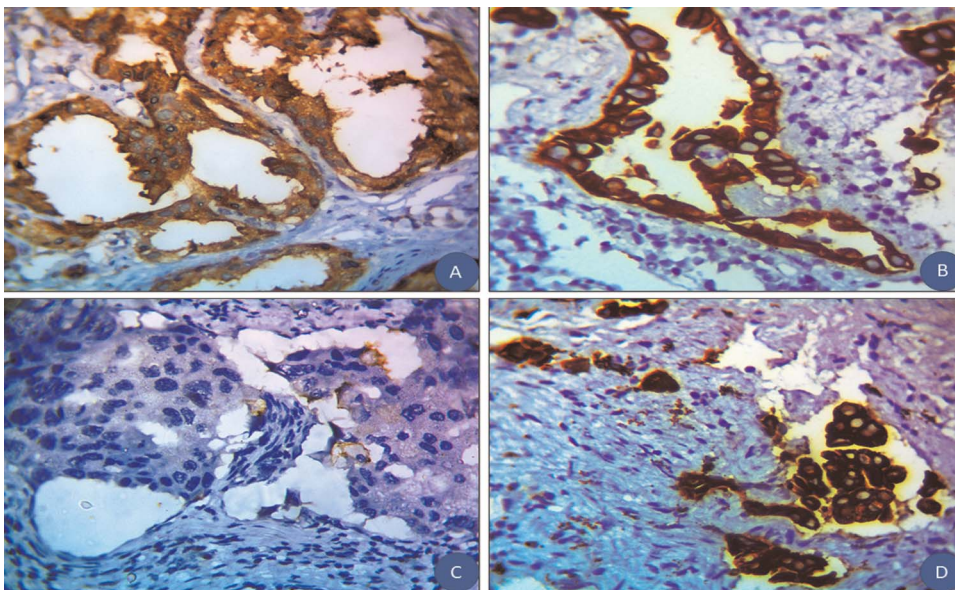


Figure 3. (A) Benign prostatic hyperplasia with a high cytoplasmic expression of DKK3 (IHC, original magnification $\times 100$). (B) Moderately differentiated prostate cancer with negative expression of DKK3 (IHC, original magnification $\times 400$). (C) Poorly differentiated prostate cancer with negative expression of DKK3 (IHC, original magnification $\times 400$). (D) Poorly differentiated prostate cancer with high expression of DKK3 (IHC, original magnification $\times 400$). DKK3 = Dickkopf homolog 3; IHC = immunohistochemistry.

3.6. Clinicopathological features and relapse

The mean follow-up duration was 44.22 months (range, 30–61 months). Sixteen of 39 patients (41%) experienced disease relapse, and 15 patients (30%) died. Pretreatment PSA and relapse were significantly associated: 6.2% of patients with PSA <10 ng/mL relapsed versus 50% of patients with PSA >20 ng/mL ($p < 0.001$). A significant association was also observed between the Gleason score and relapse: 16.7% of patients with a Gleason score of 7 relapsed versus 100% of patients with a Gleason score of 9 ($p = 0.008$). Furthermore, T stage and relapse were significantly associated with relapse in 5% of patients with T2 tumors versus in 100% of patients with T4 tumors ($p < 0.001$). Finally, the N stage and relapse were significantly associated, where 13.6% of patients with node-negative disease relapsed versus 76.6% of patients with node-positive disease ($p < 0.001$; Table 4).

3.7. Clinicopathological features and mortality

ECOG PS and mortality were significantly associated; 28.6% of patients with ECOG PS 0 died versus 58.8% of patients with ECOG PS 2 ($p = 0.002$). Similarly, the Gleason score and mortality showed a positive association, where 16.7% of patients with a Gleason score of 7 died versus 73.3% of patients with a Gleason score of 9 ($p < 0.001$). A significant association was also observed between the T stage and mortality, in which 15% of patients with T2 tumors died compared with 60% of patients with T4 tumors ($p = 0.040$). In addition, N stage and mortality were associated: 13.6% of patients with node-negative disease died versus 42.9% of patients with node-positive disease ($p = 0.025$). Finally, the M stage and mortality were significantly associated, in which 15.4% of patients with nonmetastatic disease died compared with 81.8% of patients with metastatic disease ($p < 0.001$; Table 4).

Table 4
Relation between clinicopathological features, DKK3 IHC in malignant cells, VDR Apal C/A polymorphism, and outcomes of patients with prostate cancer (n = 50).

Characteristics	Relapse				Mortality			
	Patients with prostate cancer (n = 39), n (%)	Absent (n = 23) n (%)	Present (n = 16) n (%)	p^*	Patients with prostate cancer (n = 50), n (%)	Alive (n = 35), n (%)	Died (n = 15), n (%)	p^*
Age group, yr								
<60	18 (46.2)	12 (66.7)	6 (33.3)	0.366	24 (48)	16 (66.7)	8 (33.3)	0.621
≥60	21 (53.8)	11 (52.4)	10 (47.6)		26 (52)	19 (73.1)	7 (26.9)	
Pretreatment prostate-specific antigen, ng/mL								
<10	16 (41)	15 (93.8)	1 (6.2)	<0.001 [†]	16 (32)	13 (81.2)	3 (18.8)	0.468
10–20	11 (28.2)	2 (18.2)	9 (81.8)		13 (26)	8 (61.5)	5 (38.5)	
>20	12 (30.8)	6 (50)	6 (50)		21 (42)	14 (66.7)	7 (33.3)	
ECOG PS								
ECOG 0	14 (35.9)	9 (64.3)	5 (35.7)	0.260	14 (28)	10 (71.4)	4 (28.6)	0.002 [†]
ECOG 1	19 (48.7)	9 (47.4)	10 (52.6)		19 (38)	18 (94.7)	1 (5.3)	
ECOG 2	6 (15.4)	5 (83.3)	1 (16.7)		17 (34)	7 (41.2)	10 (58.8)	
Gleason score								
Gleason 7	6 (15.4)	5 (83.3)	1 (16.7)	0.008 [†]	6 (12)	5 (83.3)	1 (16.7)	<0.001 [†]
Gleason 8	23 (59)	12 (52.2)	11 (47.8)		23 (46)	20 (87)	3 (13)	
Gleason 9	4 (10.3)	0 (0)	4 (100)		15 (30)	4 (26.7)	11 (73.3)	
Gleason 10	6 (15.4)	6 (100)	0 (0)	0.370	6 (12)	6 (100)	0 (0)	0.654
≤7	6 (15.4)	5 (83.3)	1 (16.7)		6 (12)	5 (83.3)	1 (16.7)	
>7	33 (84.6)	18 (54.5)	15 (45.5)		44 (88)	30 (68.2)	14 (31.8)	
Tumor								
T2	20 (51.3)	19 (95)	1 (5)	<0.001 [†]	20 (40)	17 (85)	3 (15)	0.040 [†]
T3	16 (41)	4 (25)	12 (75)		20 (40)	14 (70)	6 (30)	
T4	3 (7.7)	0 (0)	3 (100)		10 (20)	4 (40)	6 (60)	
Node								
N0	22 (56.4)	19 (86.4)	3 (13.6)	<0.001 [†]	22 (44)	19 (86.4)	3 (13.6)	0.025 [†]
N1	17 (43.6)	4 (23.5)	13 (76.5)		28 (56)	16 (57.1)	12 (42.9)	
Metastasis								
M0				0.001 [†]	39 (78)	33 (84.6)	6 (15.4)	<0.001 [†]
M1					11 (22)	2 (18.2)	9 (81.8)	
DKK3 IHC in malignant cells								
Low	28 (71.8)	18 (64.3)	10 (35.7)	0.307	35 (70)	23 (65.7)	12 (34.3)	0.502
High	11 (28.2)	5 (45.5)	6 (54.5)		15 (30)	12 (80)	3 (20)	
VDR Apal C/A polymorphism								
CC	11 (28.2)	7 (63.6)	4 (36.4)	0.193	13 (26)	12 (92.3)	1 (7.7)	0.082
AA	21 (53.8)	10 (47.6)	11 (52.4)		26 (52)	15 (57.7)	11 (42.3)	
CA	7 (17.9)	6 (85.7)	1 (14.3)		11 (22)	8 (72.7)	3 (27.3)	

DKK3 = Dickkopf homolog 3; ECOG PS = Eastern Cooperative Oncology Group performance status; IHC = immunohistochemistry; VDR = vitamin D receptor.

Categorical variables are expressed as number (percentage).

* χ^2 Test.

[†] $p < 0.05$ (significant).

Table 5
Relation between treatment and outcomes of patients with prostate cancer (n = 50).

Treatment	Relapse			p*	Mortality			p*
	Patients with prostate cancer (n = 39), n (%)	Absent (n = 23), n (%)	Present (n = 16), n (%)		Patients with prostate cancer (n = 50), n (%)	Alive (n = 35), n (%)	Died (n = 15), n (%)	
Local radiotherapy								
No	1 (2.6)	0 (0)	1 (100)	0.410	12 (24)	2 (16.7)	10 (83.3)	<0.001†
Yes	38 (97.4)	23 (60.5)	15 (39.5)		38 (76)	33 (86.8)	5 (13.2)	
Hormonal deprivation								
LHRH	15 (38.5)	12 (80)	3 (20)	0.035†	15 (30)	12 (80)	3 (20)	0.502
LHRH + AR blockers	24 (61.5)	11 (45.8)	13 (54.2)		35 (70)	23 (65.7)	12 (34.3)	
Neoadjuvant hormonal								
No	38 (97.4)	23 (60.5)	15 (39.5)	0.410	38 (76)	33 (86.8)	5 (13.2)	<0.001†
Yes	1 (2.6)	0 (0)	1 (100)		12 (24)	2 (16.7)	10 (83.3)	
Concurrent hormonal								
No	7 (17.9)	6 (85.7)	1 (14.3)	0.206	18 (36)	8 (44.4)	10 (55.6)	0.003†
Yes	32 (82.1)	17 (53.1)	15 (46.9)		32 (64)	27 (84.4)	5 (15.6)	
Posttreatment prostate-specific antigen, ng/mL								
≤0.5	28 (71.8)	18 (64.3)	10 (35.7)	0.307	34 (68)	24 (70.6)	10 (29.4)	1.000
>0.5	11 (28.2)	5 (45.5)	6 (54.5)		16 (32)	11 (68.8)	5 (31.2)	
Relapse					(n = 39)	(n = 33)	(n = 6)	
Absent					23 (59)	21 (91.3)	2 (8.7)	0.205
Present					16 (41)	12 (75)	4 (25)	

AR = androgen receptor; LHRH = luteinizing hormone-releasing hormone. Categorical variables are expressed as number (percentage).

*χ² Test.

†p < 0.05 (significant).

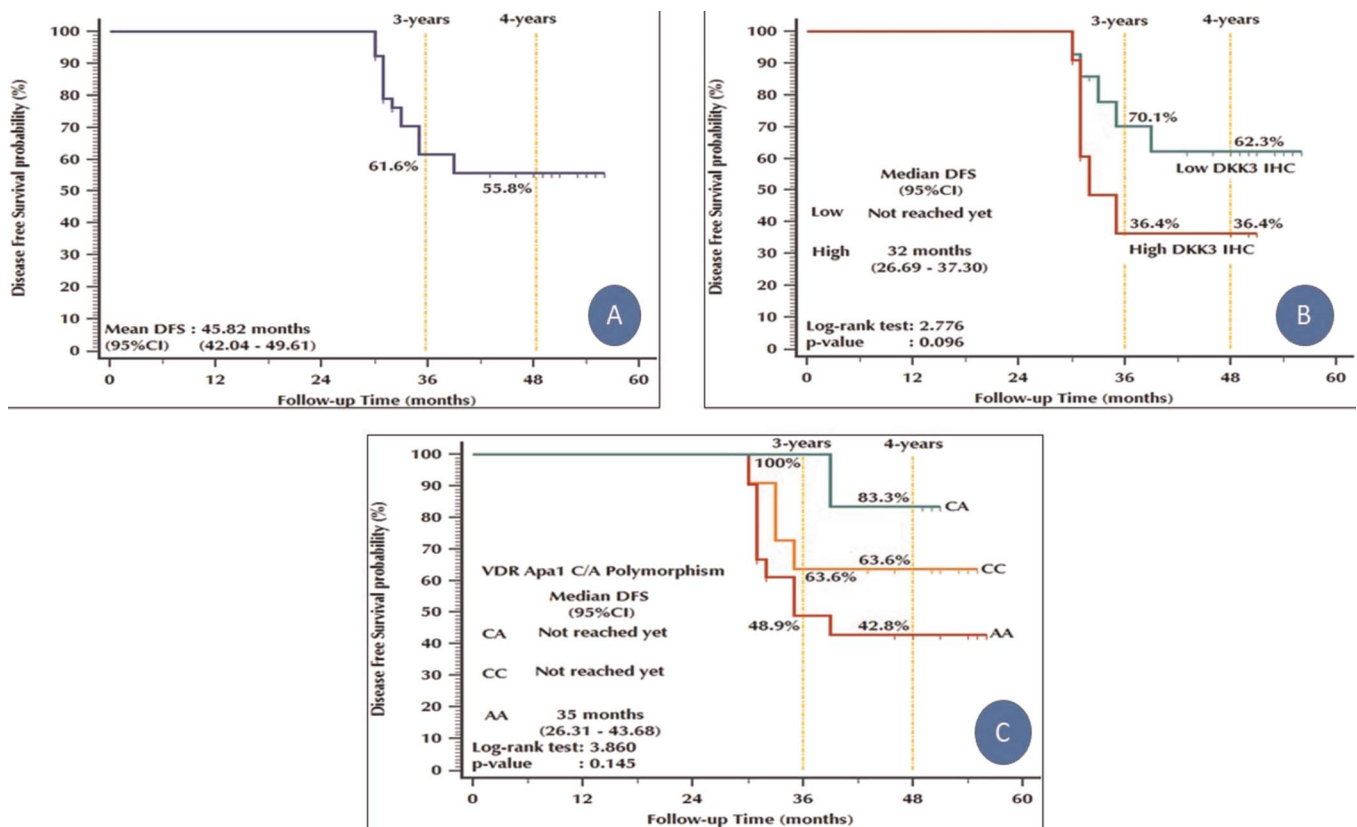


Figure 4. Kaplan-Meier plot: Disease-free survival. (A) All prostate cancer patients. (B) Stratified by DKK3 IHC in malignant cells. (C) Stratified by VDR Apa1 C/A polymorphism. DKK3 = Dickkopf homolog 3; IHC = immunohistochemistry; VDR = vitamin D receptor.

3.8. Relation between treatment and relapse

Thirty-eight patients (76%) underwent local radiotherapy. Thirty-five patients (70%) were administered a combined androgen blockade (luteinizing hormone-releasing hormone [LHRH] + AR blockers). The mean duration of hormonal treatment was 38.14 months (range, 28–56 months). Twelve patients (24%) were administered neoadjuvant hormonal therapy, and 32 patients (64%) were administered concurrent hormonal treatment. Sixteen patients (32%) showed posttreatment PSA >0.5 ng/mL. A significant association was observed between hormonal deprivation and relapse, as 20% of patients treated with LHRH relapsed versus 54.2% of patients treated with LHRH + AR blockers relapsed ($p = 0.035$; Table 5).

3.9. Relation between treatment and mortality

Local radiotherapy was positively associated with mortality: 83.3% of untreated patients died versus 13.2% of treated patients ($p < 0.001$). In contrast, neoadjuvant hormonal treatment was associated with higher mortality: 13.2% of untreated patients died versus 83.3% of treated patients ($p < 0.001$). In addition, concurrent hormonal treatment and mortality were positively associated; 55.6% of untreated patients died versus 15.6% of treated patients ($p = 0.003$; Table 5).

3.10. Kaplan-Meier plot for DFS

The 4-year DFS among group B patients was 55.8%. The 4-year DFS values among patients with low and high DKK3 expression

were 62.3% and 36.4%, respectively. The 4-year DFS values among patients with the CA, CC, and AA genotypes were 83.3%, 63.6%, and 42.8%, respectively (Fig. 4).

3.11. Kaplan-Meier plot for OS

The 5-year OS among the 50 group B patients was 28.6%. The 4-year OS values among patients with low and high DKK3 expression were 67.6% and 71.8%, respectively. In addition, the 4-year OS values among patients with the CA, CC, and AA genotypes were 70%, 91.7%, and 53.3%, respectively (Fig. 5).

4. Discussion

In addition to its role in calcium homeostasis, vitamin D affects cell growth and differentiation, and immune function, and can protect against cardiovascular disease, infections, cancer, and autoimmune diseases.^[8] Prostatic epithelial cells express VDR and 1 α -hydroxylase enzymes that are required for the synthesis and action of calcitriol.^[14]

In prostate cancer, various genes function together with individual and environmental factors to affect the development and prognosis of prostate cancer.^[17] Thus, identifying genetic risk factors is essential for early disease detection. Single-nucleotide polymorphisms may contribute to the pathogenesis of prostate cancer. Ethnic and geographic variations in the incidence and mortality of prostate cancer may result from polymorphisms in genes associated with androgen secretion and metabolism.^[18] The association of VDR polymorphisms with the risk of prostate cancer among different ethnic groups suggests that these

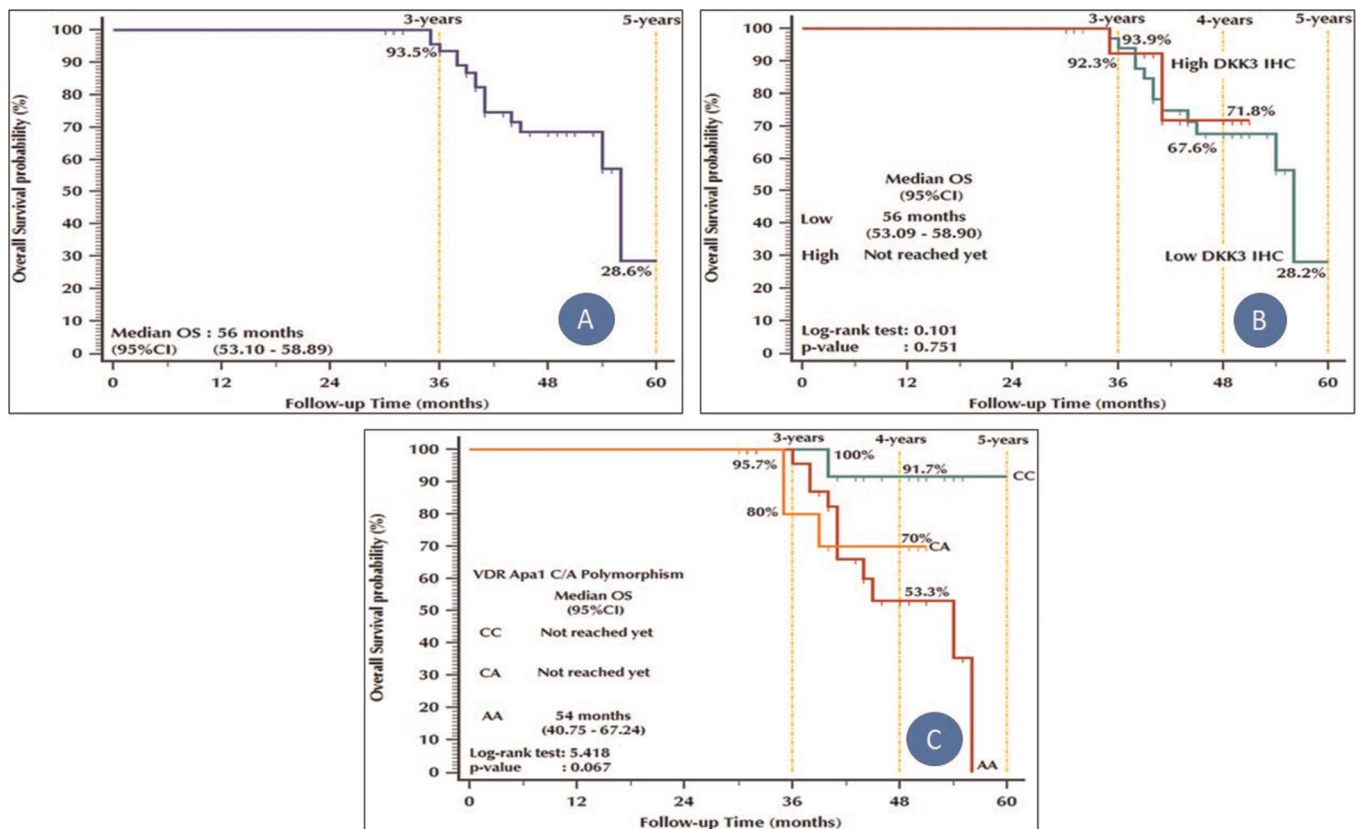


Figure 5. Kaplan-Meier plot: Overall survival. (A) Stratified by DKK3 IHC in malignant cells. (B) Stratified by VDR Apa1 C/A polymorphism. (C) All patients with prostate cancer. DKK3 = Dickkopf homolog 3; IHC = immunohistochemistry; VDR = vitamin D receptor.

polymorphisms can be used as risk markers for the disease.^[19] *VDR* is highly polymorphic, and its allele frequencies are highly variable among different races and ethnic groups.^[20]

Age is a well-established risk factor for prostate cancer, with an increased risk after 50 years of age; approximately 6 in 10 men 65 years and older present with the disease.^[19] In this study, the 2 groups of men were age-matched.

We observed a significant increase in total and direct bilirubin levels among patients with prostate cancer compared with those in controls. Causes of hyperbilirubinemia in patients with prostate cancer include obstruction of the bile duct by the primary tumor, pressure effect of enlarged lymph nodes, or infiltration of the liver.^[21] Paraneoplastic cholestasis may also be responsible for the direct effect of the primary tumor or metastasis. However, these features are extremely rare in patients with prostate cancer.^[22] Because we excluded high-volume metastasis from our study, visceral liver involvement was excluded, and thus, one possible explanation for our results is the enlarged lymph node effect.

We found a highly significant increase in PSA levels among patients with prostate cancer compared with those in controls ($p < 0.001$). An increase was observed in patient PSA levels among *CC* and *CA* genotypes compared with in *AA* genotype. Similarly, Nunes et al.^[19] reported a significant association between *VDR* polymorphisms and PSA levels. PSA expression is controlled by androgens via the AR. A link between *VDR* and AR has been suggested because prostate cells intensively respond to dihydrotestosterone in the presence of calcitriol.^[23] Furthermore, both exhibit synergistic interactions that regulate prostate cell proliferation and PSA secretion. Moreover, *VDR* polymorphisms associated with PSA levels may be useful prognostic factors. PSA concentrations >10 ng/mL were significantly associated with an increased risk of recurrence. In addition, PSA levels >2 ng/mL during the year before diagnosis increased the risk of mortality, despite treatment with radical prostatectomy.^[24]

Apal polymorphism is in intron 8 of the 3' untranslated region, a noncoding region; however, this polymorphism has not been shown to affect splicing or transcription factor binding sites and thus does not affect the quantity, structure, or function of *VDR* protein. Therefore, the polymorphism seems to be silent. However, it influences *VDR* expression and function, particularly messenger RNA stability and gene transcription. This effect of this polymorphism may be linked to other genetic variations in *VDR* itself or nearby polymorphic genes and may alter the ability of *VDR* protein to bind calcitriol or activate vitamin D response element. This influence may alter the expression of regulatory genes, such as that of cyclin-dependent kinases, that control cell division in the prostate.^[20,14]

We observed a significant difference in genotype between patients with prostate cancer and controls ($p < 0.001$). Moreover, the *CA* genotype was significantly more common in the control group than in the prostate cancer group ($p = 0.007$), but the distribution of *A* and *C* alleles did not differ between groups ($p = 0.97$). Kambale et al.^[14] found similar results and suggested that the *CA* genotype exerts a protective effect, as it is less responsive to cell proliferation. Similarly, Onen et al.^[15] found that *VDR Apal* polymorphism may confer susceptibility to sporadic prostate cancer in the Turkish (White) population and demonstrated a relationship between *C* allele carrier of *Apal* polymorphism and sporadic prostate cancer. Other studies revealed no association between the frequency of any genotype and prostate cancer.^[13,19,25,26]

No differences in *Apal VDR* polymorphisms and prostate cancer predisposition were found in White,^[27] African American,^[28] and Asian^[13] populations between cases and controls. Furthermore, *VDR Apal* polymorphism was not significantly correlated

with prostate cancer risk in 2 meta-analyses.^[26,29] However, Habuchi et al.^[30] observed a significant difference between patients with prostate cancer and female controls, and Cicek et al.^[31] found an association between *Apal* polymorphism and prostate cancer.

We found a significant association between the T stage and *VDR Apal C/A* polymorphism. However, no significant difference was found between genotypes regarding tumor differentiation, grade, Glasson score, or *DKK3* expression. Similar to our results, Suzuki et al.^[32] and Onen et al.^[15] observed no relationship between clinicopathological parameters (tumor stage, Gleason score, PSA levels) and the genotype distributions of *Apal*. Moreover, Zhang and Shan^[25] found no specific relationship between *Apal* polymorphism and prostate cancer TNM stage or Gleason score in either a dominant or recessive model. This discrepancy may be explained by ethnic variations and the different sample sizes.

5. Conclusions

VDR Apal polymorphism may play a role in the development and progression of prostate cancer. *Apal* polymorphism may help identify individuals at high risk of prostate cancer and select suitable treatment strategies. Because of the significant association between *VDR Apal* polymorphism and both PSA levels and tumor stage, *VDR Apal* polymorphism shows potential as a prognostic marker for prostate cancer in Egyptian men.

The outcomes of prostate cancer can be affected by several factors other than *VDR* polymorphism. We could not reduce selection bias through matched comparison/analysis or subgroup analysis because of the relatively small sample size. Screening for associations between genetic variants and prostate cancer prognosis requires a large sample size and long-term follow-up. Further large-scale case-control and cohort populations of different races and ethnicities with more polymorphic sites are required to confirm the association between *VDR* polymorphisms and prostate cancer development and progression.

Acknowledgments

None.

Statement of ethics

The study was approved by the Institutional Review Board of the Faculty of Medicine, Zagazig University (ZU-IRB#9237/5-1-2022). Participants provided written informed consent after the procedures, and possible hazards were explained. All procedures performed in this study involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest statement

No conflict of interest has been declared by the authors.

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Data availability statement

Data are available upon a reasonable request.

Author contributions

AA, AZE-a, SH: Conception;
 AA, SH, SA, MKE, HML, MH, AZ-a: Interpretation or analysis of data;
 SH: Preparation of the manuscript;
 All authors: Revision for important intellectual content;
 AA, AZE-a, SH: Supervision.

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