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Prognostic Significance of PD-L1 and PTEN Expression in Prostatic Cancer

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Abstract

Background: Programmed death- ligand 1(PD-L1) acts as an immune checkpoint inhibitor. Phosphatase and tensin homolog (PTEN) is a somatically mutated tumor suppressor gene in numerous types of human cancer. The current study aimed to assess the prognostic value of PD-L1 and PTEN expression in prostatic cancer patients, as well as their relationship with the clinicopathological features of the disease.

Method: A total of 55 needle biopsy specimens were retrospectively diagnosed as prostatic adenocarcinoma. Immunohistochemical staining with PD-L1 and PTEN were evaluated in all the cases. The patients were followed up for 5 years in order to detect disease recurrence and survival.

Results: PD-L1 expression in Prostate cancer was positively correlated with high prostatic specific antigen (PSA), higher Gleason score, advanced stage, higher tumor relapse, and worse disease-free and overall survival ($P < 0.001$). PTEN loss was significantly associated with high PSA, higher Gleason score < 7, advanced tumor stage, tumor relapse, and worse disease-free and overall survival ($P < 0.001$). We observed a significant negative correlation between PTEN and PD-L1.

Conclusion: PDL-1 and PTEN are prognostic markers for prostate cancer, which can differentiate between the patients who are at a high risk of disease progression and may successively provide novel targeted therapies.

Keywords: Prostatic neoplasms, PD-L1, PTEN, Immunohistochemistry, Prognosis

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Introduction

Prostate cancer (PC) is a prevalent neoplasm among men worldwide.[1] In Egypt, PC has represented about 4.27%, according to the national population-based program.[2] Disease recurrence after surgery and progression to castration-resistant prostate cancer (CRPC) is a major challenge; therefore, detecting new molecules is of great importance to overcome this problem.[3]

Programmed cell death-ligand1 (PD-L1) is an immune checkpoint inhibitor; activation of its pathway allows tumors to escape the host's

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immune system.[4] Blockade of this checkpoint can be used in cancer immunotherapy.[5] PDL-1 increases in several epithelial malignancies.[6] In prostatic cancer, PDL-1 is associated with clinical progression to CRPC; thus, it is effective in immunotherapy of these cases.[7]

Phosphatase and tensin homolog (PTEN) is a tumor suppressor gene located on chromosome 10. It has numerous functions in cell cycle, metabolism, and cell death.[8] PTEN acts by inhibiting PI3K/AKT pathway, and is mutated in many human cancers as kidney, breast, lung, bladder, and prostate.[9] Decreased PTEN expression leads to the increased risk of PC progression and recurrence.[10]

We conducted this study to detect the immunoexpression of PD-L1 and PTEN in PC, observe their relationship with prognosis and clinicopathologic features of the disease, and develop a new method to describe the relationship between PDL-1,PTEN, and immunotherapy of high-risk and CRPC patients.

Patients and Methods

This retrospective study was carried out on 55 cases of PC (30 cases post radical prostatectomy and 25 cases post transurethral resection) selected from the archives of the Pathology Department, Medicine College, Zagazig University Hospitals, Egypt, between March 2015 and November 2018.This study has been approved by the institutional ethics committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All the subjects signed the informed consent prior to the therapy; however, for the retrospective review of data with less than the minimal risk to the patients, no consent was required by the ethics committee. The clinical data as age and follow-up-related data were collected retrospectively from the archives of Clinical Oncology and Nuclear Medicine Department. Histopathology characteristics were confirmed by blinded review of the original pathology slides. Gleason score according to WHO Classification was used.[11]

Immunohistochemistry

Thick sections of 5 µm from paraffin blocks were placed on positively charged slides, deparaffinized in xylene, and rehydrated in ethyl alcohol. Antigen retrieval was performed by boiling the sections in citrate buffer (pH 6.0) for 20 min, which were washed with phosphate-buffered saline.The specimens were incubated for two hours at 37°C with rabbit monoclonal anti-PDL-1 (diluted 1/100, Catalog Number: ACI 3171, BioCare Medical) and rabbit polyclonal anti-PTEN antibody(diluted 1/200, BioCare Medical). Normal tonsils and normal prostatic

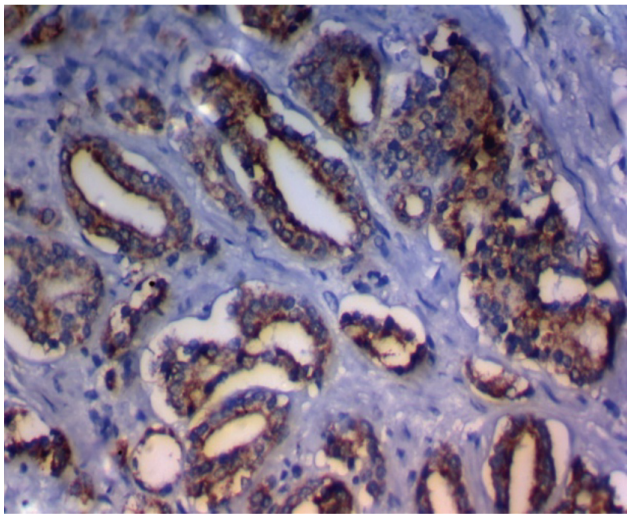

Figure 1. This figure shows moderate PDL-1expression in prostatic adenocarcinoma, Gleason Score 7 (3+4) (original magnification ×400).
PDL-1: Programmed death- ligand 1

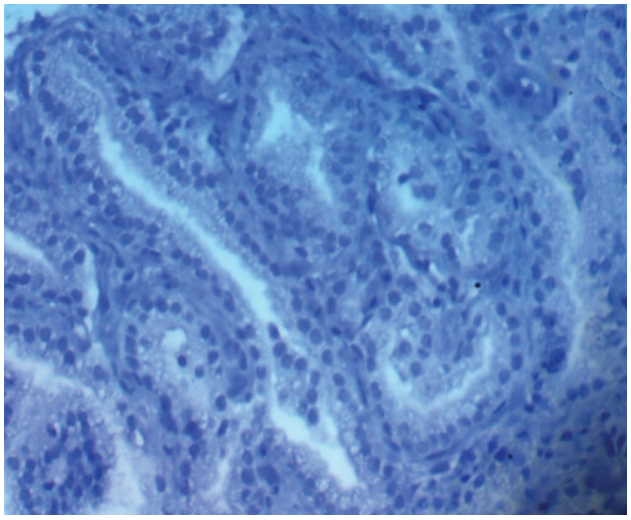

Figure 2. This figure shows negative PDL-1expression in prostatic adenocarcinoma, Gleason Score 6 (3+3) (original magnification ×400).
PDL-1: Programmed death- ligand 1

acini were used as positive controls for PD-L1 and PTEN, respectively.[12]

Evaluation of PD-L1 and PTEN staining

We evaluated the markers expression in tumor cells only.

PD-L1: cytoplasmic or membranous positive staining were scored as negative (0), weak (1), moderate (2), and strong (3).[13]

PTEN: Cytoplasmic and nuclear staining was considered positive for PTEN. The cases with PTEN protein loss decreased or were entirely negative across >10% of tumor cells.[14]

Treatment regimen

The low-risk group was treated by either prostatectomy or radical irradiation. We treated the intermediated-risk group through hormonal therapy for 6 months, followed by prostatectomy or radical irradiation. The high-risk group was treated via neoadjuvant hormonal therapy, then radical irradiation, and followed by adjuvant hormonal therapy for 2-3 years.

Statistical analysis

The trend of change in the distribution of frequencies was studied using the chi-square test for trend. Disease-free survival (DFS) and overall survival (OS) stratification was performed using the method of Kaplan-Meier plot. All the tests were two-sided. We considered *P* value < 0.05 to be significant. All the statistics were performed

Table 1. Patients' characteristics

	N=55	%
Age (years)		
<50	11	20
50 – 59	15	27.3
>60	29	52.7
PSA:		
<10	13 (23.6)	
>10	42 (76.4)	
Gleason score		
< 7	9	16.4
=7	16	29
>7	30	54.6
Gleason group (WHO 2016)		
1(< 6)	9	16.4
2(3+4)	10	18.2
3(4+3)	6	10.9
4 (8)	11	20
5(>8)	19	34.5
Tumor stage		
pT1	12	21.8
pT2	8	14.5
pT3	15	27.3
pT4	20	36.4
Disease-free survival		
Mean ± SD	37.49 ± 25.68	
Median (Range)	30 (8- 89)	
Overall survival (n=)		
Mean ± SD	42 ± 26.3	
Median (Range)	35 (11 – 100)	
Outcome		
Alive	47	85.5
Dead	8	14.5

SD: Standard deviation

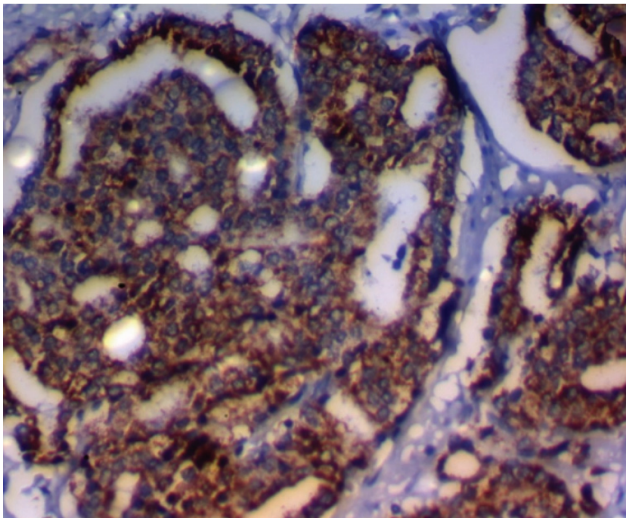

Figure 3. This figure shows strong PDL-1expression in prostatic adenocarcinoma, Gleason Score 8 (4+4) (original magnification ×400).
PDL-1: Programmed death- ligand 1

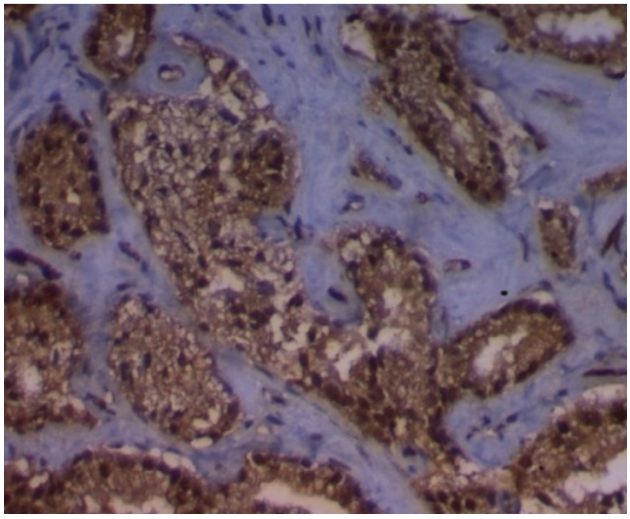

Figure 4. This figure shows moderate PTEN expression in prostatic adenocarcinoma, Gleason Score 7 (4+3) (original magnification ×400).
PTEN: Phosphatase and tensin homolog

Table 2. Relationship between PDL-1 and PTEN levels and disease-specific characteristics

	PDL-1			PTEN		
	Low N= 14 (25.5%)	High N= 41 (74.5%)	*P* value	Low N= 31 (56.4%)	High N= 24 (43.6%)	*P* value
Age group						
<50 (n=11)	3 (27.3)	8 (72.7)	0.85	6 (54.5)	5 (45.5)	0.946
50 – 59 (n=15)	3 (20)	12 (80)		9 (60)	6 (40)	
>60 (n=29)	8 (38.1)	21 (61.9)		16 (55.2)	13 (44.8)	
PSA						
<10	9 (69.2)	4 (30.8)	<0.001*	1 (7.7)	12 (92.3)	<0.001*
>10	5 (11.9)	37 (88.1)		30 (71.4)	12 (28.6)	
Tumor stage						
pT1	10 (83.3)	2 (16.7)	<0.001*	0 (0)	12 (100)	<0.001*
pT2	3 (37.5)	5 (62.5)		1 (12.5)	7 (87.5)	
pT3	1 (6.7)	14 (93.3)		11 (73.3)	4 (26.7)	
pT4	0 (0)	20 (100)		19 (95)	1 (5)	
Gleason score						
<7 (n = 9)	8 (88.9)	1 (11.1)	<0.001*	1 (12.5)	8 (87.5)	<0.001*
7 (n = 16)	5 (31.2)	11 (68.8)		6 (37.5)	10 (62.5)	
>7 (n = 30)	1 (3.3)	29 (96.7)		24 (80)	6 (20)	

PDL-1: Programmed death- ligand 1; PTEN: Phosphatase and tensin homolog; PSA: Prostatic specific antigen; *P* value <0.001; *: Highly significant

with SPSS 22.0 for windows.

Results

Table 1 represents the patients' characteristics. Table 2 depicts the relationship between PDL-1 and PTEN levels in PC and disease-specific characteristics.

Regarding PDL-1 expression, 25.5% of the cases showed low PDL-1, while 74.5% showed high PDL-1 (Figures 1-3). Meanwhile, concerning PTEN expression, 56.4% of the studied cases indicated low PTEN, whereas 43.6% showed high PTEN (Figures 4-6).

High PD-L1 and low PTEN were positively correlated with high prostatic specific antigen, advanced T stage (100%, 95%), and high Gleason score >7 (96.7%, 80%). However, we found non-significant relationships between PDL-1 and PTEN levels and the age groups.

The cases with high PDL-1 and low PTEN

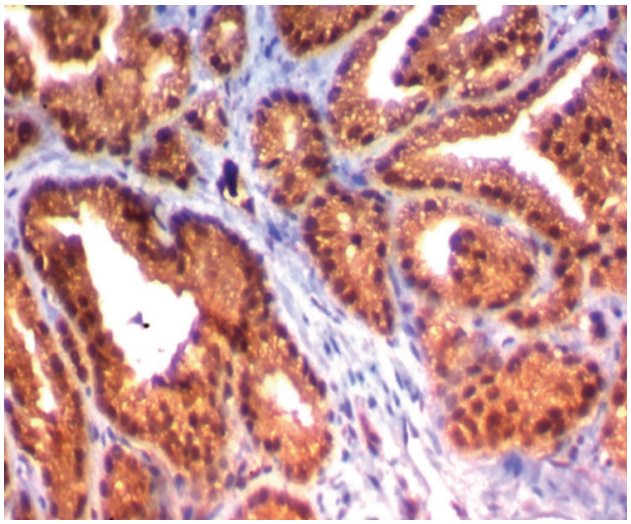

Figure 5. This figure shows strong PTEN expression in prostatic adenocarcinoma, Gleason Score 7 (3+4) (original magnification ×400).
PTEN: Phosphatase and tensin homolog

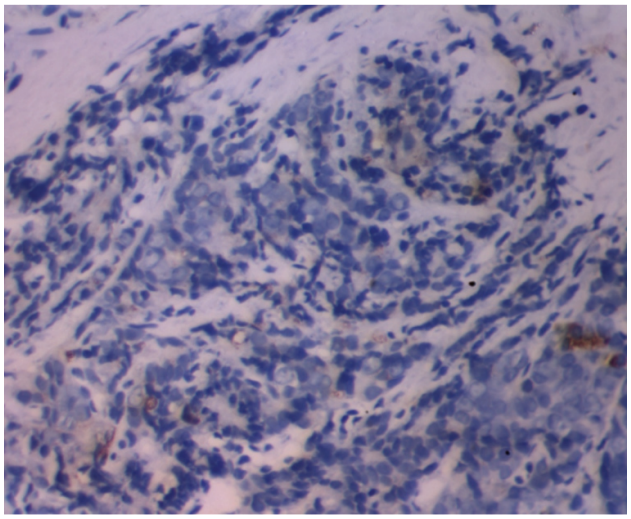

Figure 6. This figure shows negative PTEN expression in prostatic adenocarcinoma, Gleason Score 10 (5+5) (original magnification ×400).
PTEN: Phosphatase and tensin homolog

Table 3. Relationship between PDL-1 and PTEN levels and the patients' survival

	PDL-1 level			PTEN		
	Low N= 14 (25.5%)	High N= 41 (74.5%)	*P* value	Low N= 31 (56.4%)	High N= 24 (43.6%)	*P* value
Relapse (45)						
Free	37 (97.4)	1 (2.6)	<0.001#	10 (26.3)	28 (73.7)	0.032#
Present	1 (14.3)	6 (93.8)		5 (71.4)	2 (28.6)	
Outcome						
Alive	40 (85.1)	7 (14.9)	<0.001#	17 (36.2)	30 (63.8)	0.016#
Dead	1 (12.5)	7 (85.7)		7 (85.7)	1 (12.5)	
DFS						
Mean ± SD	43.2±26.1	20.8±15.5	0.006¥	23.6±20	48.3±24.6	<0.001¥
Median (range)	45 (8-89)	12 (8 – 55)		17 (11 – 80)	50 (11-100)	
OS						
Mean ± SD	47.7 ± 26.7		0.008¥	27.3±20.5	53.5±24.8.	<0.001¥
Range	50 (11-100)	17 (11-60)		12.5 (8-78)	48 (9-89)	

PDL-1: Programmed death- ligand 1; PTEN: Phosphatase and tensin homolog; DFS: Disease-free survival; OS: Overall survival; SD: Standard deviation; *P* value <0.005; #: Significant, *P* value <0.001; ¥: Highly significant

expression had a higher incidence of relapse after the therapy, in addition to poor DFS and OS rates ($P < 0.001$)(Table 3 and Figures 7-10).

We observed a strong negative correlation between PDL-1 and PTEN among the studied patients ($P < 0.001$) (Table 4).

High PDL-1 was found to increase the risk of mortality by 40 folds (4.24 – 377.1) with 95% confidence interval (CI), while low PTEN increased the risk by 12.35 folds (1.4 – 109.1) with 95% CI (Table 5).

Discussion

In the current study, high PD-L1 expression was detected in 41/55 (74.5%) of the PC cases. Gevesleben et al.[15] demonstrated high PDL-1 in 109/209 (52.2%) of cases, similar to the studies by Masari et al.[16] and Li et al.,[13] where PDL-1 expression was reported in 50% and 49.6% of the cases, respectively.

However, Xian et al.[17] documented that PDL-1 was positive in only 50/279 (17.9%) of the studied PC patients. Similarly, another study by Haffner et al.[18] reported PDL-1positive in 7.7% of the cases. Herein, we found a correlation

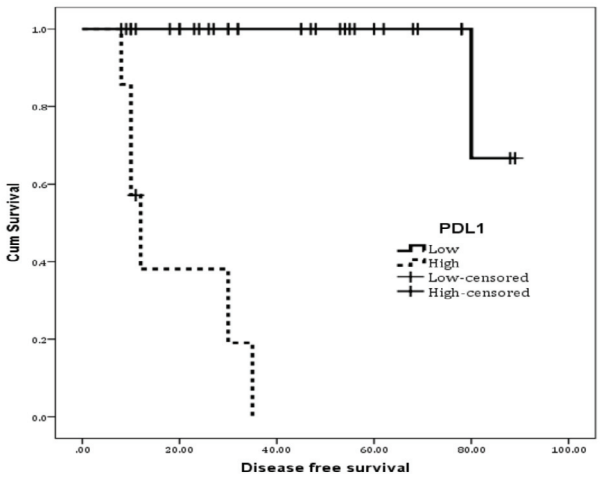

Figure 7. This figure shows the Kaplan Meier plot displaying the relationship between PDL-1 level and disease-free survival (*P* for mantel-cox < 0.001).
Cum: Cumulative; PDL-1: Programmed death- ligand 1

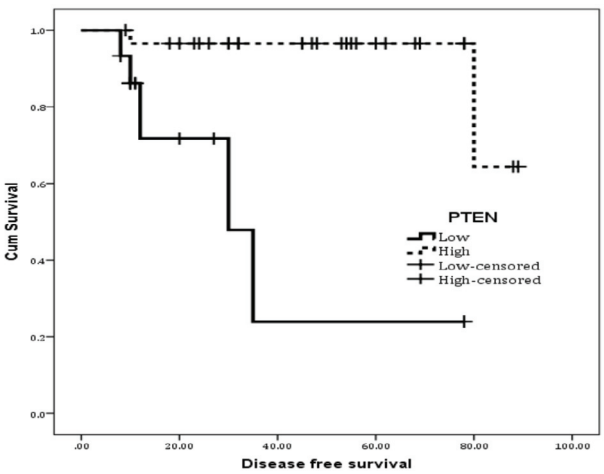

Figure 8. This figure shows the Kaplan Meier plot displaying the relationship between PTEN and disease-free survival.
Cum: Cumulative; PTEN: Phosphatase and tensin homolog

Table 4. Correlation between PDL-1 and PTEN markers among the studied patients

	PDL-1		Test	
	Low N= (%)	**High N= (%)**	**X^2**	***P* value**
PTEN				
Low	12 (50)	12 (50)	Fisher	<0.001*
High	29 (93.5)	2 (6.5)		
Phi	**-0.496**		***P***	**<0.001***

PDL-1: Programmed death- ligand 1; PTEN: Phosphatase and tensin homolog; *P* value <0.001;*: Highly significant

between high PDL-1 and high graded PC, and advanced stage; our findings are in line with those documented by Sharma et al.[19] and Ness et al.[20]

Kaplan–Meier survival analysis of PD-L1 expression confirmed that high PD-L1 expression was associated with significantly reduced DFS and OS, which increased the risk of mortality by 40 folds (4.24 – 377.1) with 95% CI; it is in accordance with another study by Petitprez et al.[21] On the other hand, Sharma et al.[19] failed to show such associations.

In the current study, we detected low PTEN in 56.3% of the cases. Noh. et al.[22] reported low PTEN in 76.5% (52/68) of the PC cases with increased disease recurrence. Lotan et al.,[23] in their cohort of 217 PC cases, reported PTEN loss in 75% of the subjects. We also found that low PTEN was strongly correlated with high graded prostatic carcinoma, which is in agreement with two other papers.[24, 25]

In the present work, Kaplan–Meier survival analyses revealed that low PTEN expression was associated with shorter biochemical recurrence and shorter survival time, as in other studies.[26-28] Not only can prognostic value be provided by PTEN, but it can also be diagnosed as reported by Giannico et al.[29]

We observed a strong negative correlation between PDL-1 and PTEN among the studied patients as PTEN deficiency was associated with an immunosuppressive state that increased the expression of PDL-1; this result is similar to that of other studies, indicating that PTEN loss correlates with a reduction in T cell inflammatory responses and worse outcomes with anti-PD-1 immunotherapy.[30, 31]

Jamaspishvili et al.[32] reported that PTEN influences immune response to tumor progression and has a role in predicting which patients will respond to promising immunotherapies.

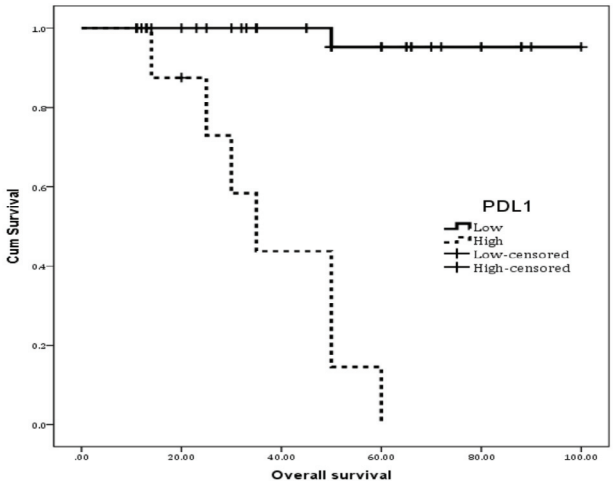

Figure 9. This figure shows the Kaplan Meier plot displaying the relationship between PDL-1 level and overall survival (*P* for mantel-cox < 0.001).
Cum: Cumulative; PDL-1: Programmed death- ligand 1

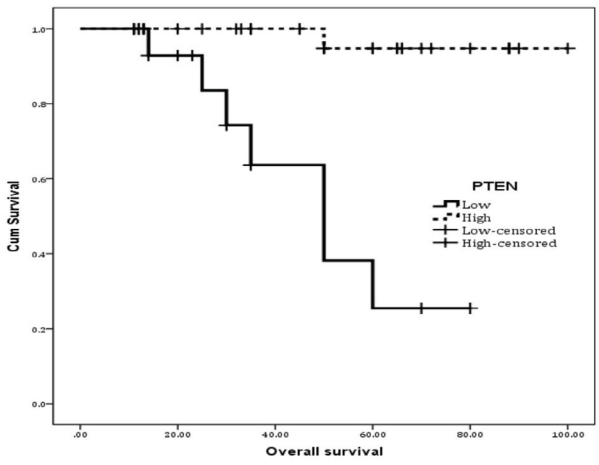

Figure 10. This figure shows the Kaplan Meier plot displaying the relationship between PTEN and overall survival (*P* for mantel-cox < 0.001).
Cum: Cumulative; PTEN: Phosphatase and tensin homolog

Table 5. Relationship between PDL-1 and PTEN levels in the studied patients and their outcome

Markers	Dead N=8	Alive N=47	*P* value	OR	95% CI
PDL-1					
Low	1 (2.4)	40 (97.6)	<0.001*	40	4.24 – 377.1
High	7 (50)	7 (50)			
PTEN					
Low	7 (29.8)	17 (70.8)	0.016*	12.35	1.4 – 109.1
High	1 (3.2)	30 (96.8)			

OR: Odds ratio; CI: Confidence interval; PDL-1: Programmed death- ligand 1; PTEN: Phosphatase and tensin homolog; *P* value <0.001; *: Highly significant

Regarding the limitation in this paper, we could mention that it was a retrospective study; the sample size was relatively small and the evaluation of expression of PTEN and PDL-1 markers was performed only by immunohistochemistry with no genetic assessments.

Conclusion

Low PTEN and high PDL-1 were found to be poor prognostic factors that could improve early detection and prognosis and provide targets for therapeutic interventions. Low PTEN status could select patients for immunotherapy in PC patients through checkpoint blockade.

Conflict of Interest

None declared.

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