Expression of PTEN protein in prostatic adenocarcinoma

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Abstract

Background: PTEN protein is a new biomarker for identifying poor prognosis in prostatic adenocarcinoma. **Objective:** To evaluate the expression of PTEN protein in prostatic adenocarcinoma and assess the correlation between expression of PTEN protein with other clinicopathological characteristics in prostatic adenocarcinoma. **Materials and methods:** A descriptive analysis of 121 radical prostatectomy specimens diagnosed with prostatic adenocarcinoma at Binh Dan Hospital from January 2022 to December 2023. Interpret H&E and PTEN immunohistochemistry (IHC) slides. Analyse data and the correlation between clinicopathological and PTEN expression variables. **Results:** 72.7% of cases showed intact PTEN protein by IHC, while the rates of homogeneous and heterogeneous PTEN loss were 14.1% and 13.2%, respectively. **Conclusion:** Expression of PTEN protein was significantly associated with the 2019 International Society of Urological Pathology (ISUP) grade groups; however, there was no significant correlation between PTEN expression and other clinicopathological characteristics.

Keywords: *prostatic adenocarcinoma, PTEN protein, radical prostatectomy.*

1. BACKGROUND

Prostate cancer (PCa) is one of the most commonly diagnosed cancers in men and its incidence rate increases with age. In Vietnam, PCa incidence rate is the 5th highest and mortality rate is the 6th highest among cancers in men. Over the last two decades, PCa incidence and mortality rate have tended to increase, posing many challenges for the diagnosis and treatment of PCa. Recent advancements in molecular biomedicine have led to the emergence of numerous new molecular biomarkers, aimed at facilitating clinical decision-making for more reliable diagnosis, treatment selection, and risk stratification in PCa. One such biomarker is the PTEN protein. Many studies have demonstrated the correlation between loss of PTEN protein expression with poor prognosis in patients with PCa. Loss of PTEN protein expression can help predict time to development of metastasis, prostate cancer-specific mortality, castration-resistant prostate cancer and response to androgen deprivation therapy after radical prostatectomy [1]. In low-risk patients, PTEN loss adds prognostic value to Gleason score, PSA, Ki-67 and extent of disease [2]. Moreover, in patients with clinically localized PCa treated by prostatectomy, PTEN loss is an independent clinicopathological factor of recurrence [3].

There have been many studies of evaluation of PTEN protein expression by immunohistochemistry (IHC) in PCa; however, no research on this topic has been published in the South of Vietnam. We conducted this study with the aim of evaluating and applying IHC staining for the PTEN protein in conjunction with pathological interpretation for the diagnosis of PCa.

2. MATERIALS AND METHODS

2.1. Materials: 121 radical prostatectomy specimens diagnosed with prostatic adenocarcinoma at Binh Dan Hospital from January 2022 to December 2023.

2.2. Methods: This study is a descriptive analysis. Collecting patients' age, preoperative serum PSA level, histopathological characteristics of PCa, intepreting H&E and PTEN IHC slides, analysing data and the correlation between clinicopathological and PTEN expression variables.

Procedure:

- Select from each case three distinct regions of the tumour tissue to construct a tissue microarray (TMA) specimen following a predetermined map: core (1) includes normal glands (internal positive control); core (2) includes Gleason primary tumour grade and core (3) includes Gleason secondary tumour grade.

- The TMA slides were stained with PTEN (SP218) monoclonal primary antibody (VENTANA Roche) by VENTANA BenchMark GX instrument.

- Evaluate PTEN expression:

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+ **Internal positive control:** surrounding benign glands and/or stroma.

+ **PTEN intact:** if > 90% of sampled tumour glands expressed cytoplasmic and nuclear staining.

+ **PTEN loss:** if the intensity of cytoplasmic and nuclear staining was markedly decreased or entirely negative across > 10% of tumour cells compared with internal positive control.

- ***Heterogeneous PTEN loss:*** if PTEN was loss in < 100% of the tumour cells sampled in a given core.
- ***Homogeneous PTEN loss:*** if the core showed PTEN loss in 100% of sampled tumour glands.

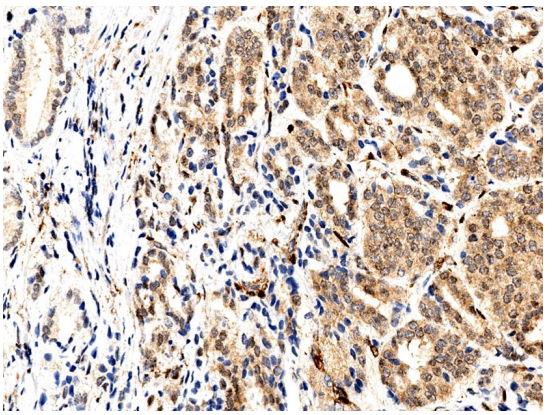

Figure 1. Grade group 2: PTEN intact (200x)

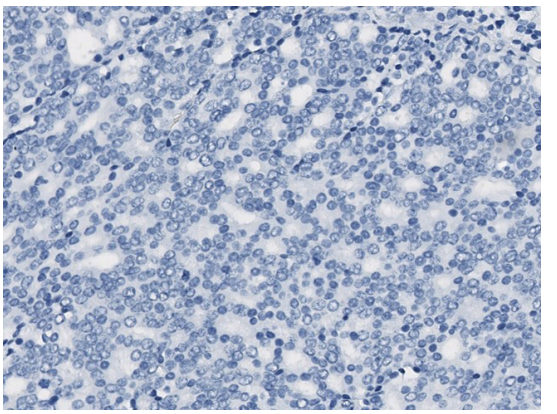

Figure 2. Grade group 3: homogeneous PTEN loss (200x)

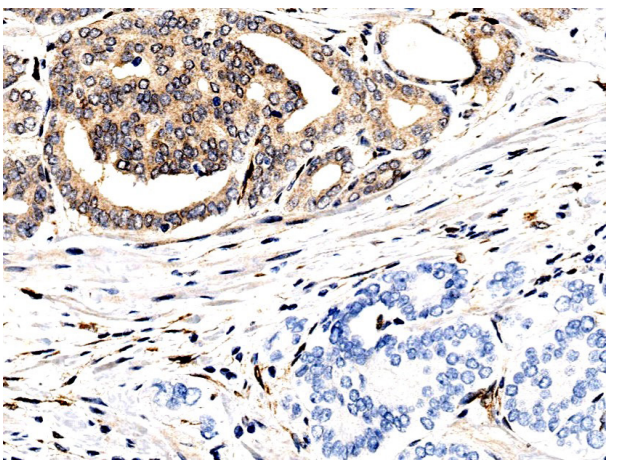

Figure 3. Grade group 3: heterogeneous PTEN loss (200x)

Statistical analysis:

- Data were analysed with Stata software, version 17.

- Qualitative variables were described through percentage and frequency.

- Quantitative variables were described with mean ± standard deviation (normal distribution) or median and interquartile range (skewed distribution).

- When assessing the correlation between PTEN protein expression and other clinicopathological characteristics, we conducted an analysis between the two groups, "PTEN intact" and "PTEN loss", to avoid the small numbers within the subgroups of "Homogeneous PTEN loss" and "Heterogeneous PTEN loss".

- $p < 0.05$ considered statistically significant in all tests.

3. RESULTS

Some clinicopathological characteristics of the patients were shown in **Table 1**.

Table 1. Clinicopathological characteristics

Mean age	68.2 ± 7.2
Median PSA level (ng/ml)	29.5
	n (%)
ISUP 2019 grade group	
1	8 (6.6)
2	23 (19.0)
3	52 (43.0)
4	12 (9.9)
5	26 (21.5)
Seminal vesicle invasion (n = 120)	
Unilateral	14 (11.7)
Bilateral	39 (32.5)
No	67 (55.8)
Lymph node metastasis (n = 51)	
Yes	9 (17.6)
No	42 (82.4)
Positive surgical margin (n = 119)	
Yes	61 (51.3)
No	58 (48.7)

In our study, the PTEN intact rate was 72.7%. The homogeneous and heterogeneous PTEN loss rate were 14.1% and 13.2%, respectively.

100% of cases in grade group 1 expressed PTEN protein. Conversely, grade group 5 showed the lowest rate of expression of PTEN protein, with 57.7%.

None of cases in grade group 1 exhibited homogeneous PTEN loss. Homogeneous PTEN loss rate was highest in grade group 4 (25%).

There were no cases in grade group 1 and grade group 4 showed heterogeneous PTEN loss, while highest heterogeneous PTEN loss rate was observed in grade group 3 and grade group 5 (19.2%).

PTEN expression by IHC remained significantly associated with ISUP 2019 grade groups (Fisher's exact, p=0.023), all the cases in grade group 1 showed PTEN intact, but this rate decreased drastically in grade group 5, where only 57.7% of cases demonstrated expression of the PTEN protein.

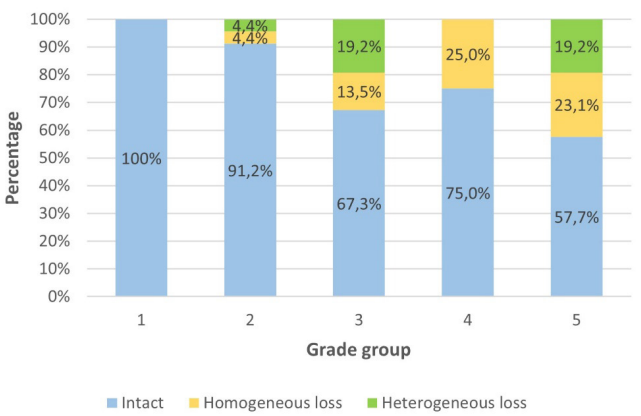

Figure 4. Expression of PTEN protein by ISUP 2019 grade groups

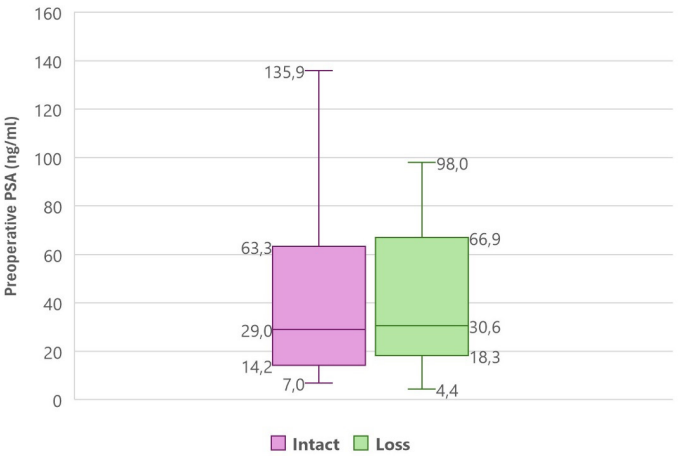

Figure 5. Preoperative serum PSA level between PTEN intact and PTEN loss group

The median preoperative serum PSA level of PTEN intact group and PTEN loss group were 29.0 and 30.6 ng/ml, respectively. The difference of preoperative serum PSA level between PTEN intact and PTEN loss group was not statistically significant (p=0.859).

There were no significant correlations between PTEN protein expression and seminal vesicle invasion (p=0.148), lymph node metastasis (p=0.393) and positive surgical margin (p=0.393) (**Table 2**).

Table 2. Correlation between PTEN protein expression and some adverse pathological characteristics

Characteristics	Intact	Loss	Total	p value
	n (%)	n (%)	n (%)	
Seminal vesicle invasion (n = 120)				
Unilateral	10 (71.4)	4 (28.6)	14 (11.7)	0.148 (χ^2)
Bilateral	24 (61.5)	15 (38.5)	39 (32.5)	
No	53 (79.1)	14 (20.9)	67 (55.8)	
Lymph node metastasis (n = 51)				
Yes	5 (55.6)	4 (44.4)	9 (17.6)	0.393 (Fisher's exact)
No	28 (66.7)	14 (33.3)	42 (82.4)	
Positive surgical margin (n = 119)				
Yes	42 (68.8)	19 (31.2)	61 (51.3)	0.393 (χ^2)
No	44 (75.9)	14 (24.1)	58 (48.7)	

4. DISCUSSION

The PTEN protein expression rate in this study was 72.7%, which shows similarity with other studies. The homogeneous PTEN loss rate was 14.1% and the heterogeneous PTEN loss rate was 13.2%, which are relatively consistent with the findings reported by Haney (2020), Lotan (2016), and Ahearn (2016) [4-6]. However, the homogeneous and heterogeneous PTEN loss rate observed in the studies of Zhang (2022) and Lotan (2017) differed from our results. These two authors reported a very low rate of heterogeneous loss, at only 2%, whereas the rate of homogeneous loss was 10 to 13 times higher, at 20% in Lotan's (2017) study and 26.3% in Zhang's study [7], [8].

PCa is a highly heterogeneous tumour; however, in the study by Lotan et al. (2017), the authors utilised only one 0.6 mm diameter TMA core for each case, this may account for the significantly lower rate of heterogeneous PTEN loss observed in the authors' study compared to other studies that employed 3 to 4 TMA cores for each case, including this study [8].

In the study by Zhang et al., the rate of homogeneous PTEN loss was over 10 times greater than that of heterogeneous loss, which may be attributed to the fact that Zhang's patients were

cases of metastatic PCa. The findings of Zhang align with previous studies indicating that cases at the advanced stage with distant metastasis are associated with mutations resulting in the loss of PTEN protein expression, and homogeneous PTEN loss has a higher prognostic value than heterogeneous loss [7].

The results of our study, along with those of Lotan and Haney, show a similarity in that the highest rate of PTEN intact is found in the grade group 1, which subsequently declines in the following grade groups, reaching the lowest rate in the grade group 5. All three studies reported statistically significant differences in PTEN protein expression rates among the ISUP 2019 grade groups. While there is a similarity in PTEN intact rates across the ISUP 2019 grade groups with the aforementioned authors, the intact rate in grade group 1 and 2 in our study were both above 90%, surpassing those reported by Lotan and Haney (approximately 86% in grade group 1 and around 75% in grade group 2). This difference may be attributable to the differing proportions of grade group 1 and 2 cases within our study population, which comprised approximately 25% of cases in grade group 1 and 2, whereas Lotan and Haney's studies reported over 75%.

In contrast, Zhang et al. reported that among cases with a Gleason score < 8 (grade group 1, 2, and 3), there were 7 cases (58.3%) that expressed PTEN protein, while 5 cases (41.7%) demonstrated PTEN loss. In the group with a Gleason score ≥ 8 (grade group 4 and 5), 140 cases (72.5%) showed PTEN intact, whereas 53 cases (27.5%) exhibited PTEN loss [7]. These results are contrary to our findings as well as those of Lotan and Haney, where the rate of PTEN intact in grade group 4 and 5 were found to be higher than in grade group 1, 2, and 3. Additionally, Zhang did not observe any correlation between PTEN protein expression and the grading groups [4, 8]. Additionally, Zhang did not observe any correlation between PTEN expression by IHC and the ISUP 2019 grade groups. The underlying reason for this issue may be that Zhang's study population comprised cases of metastatic cancer, which is a common characteristic among higher grade groups. Consequently, this leads to differences between the grade groups, as cases with Gleason score < 8 accounted for only approximately 6% of the study population, this indirectly results in variations in PTEN protein expression rates between the grade groups.

There was no correlation observed between PTEN protein expression and preoperative serum PSA levels. However, the study by Antonarakis indicated that preoperative serum PSA levels in PTEN loss group was significantly lower than that of PTEN intact group. Conversely, Lotan's study demonstrated that increasing frequency of PTEN loss was seen in association with increasing higher preoperative serum PSA levels.[8, 9] Some factors contributing to the differences between the results of our study and those of the two aforementioned authors may include differences in sample size and ethnicity.

Our study observed a PTEN intact rate of 55.6% in cases with lymph node metastasis, a figure that is similar to the study by Lotan et al. in 2017, which reported an expression rate of 51.3% [8]. However, we did not find any correlation between PTEN protein expression and lymph node metastasis, whereas Lotan's study showed a correlation between these two variables. This discrepancy may be attributed to the limited number of cases examined in our study, with only 51 cases compared to 5386 cases in Lotan's study, which may not be sufficient to draw representative conclusions.

The rate of PTEN intact in cases with positive surgical margin in our study was 68.8%. Other authors have also reported similar results, with the rate of 68% in Lotan's study, 66% in Mithal's research, and 71.9% in Haney's study [1, 4, 8]. Mithal's, Haney's, and our study did not observe any correlation between these two variables, albeit Lotan et al. reported a correlation between them.

In our study, the rate of PTEN intact in cases of seminal vesicle invasion was 64.1%, whereas Mithal et al. reported a rate of 15% [1]. The distinction in these findings may be attributable to differences in sample size and ethnicity between our study and that of Mithal.

5. CONCLUSION

The PTEN protein expression rate in prostatic adenocarcinoma in our study was 72.7%. The expression rate was highest in grade group 1 and lowest in grade group 5. The homogeneous and heterogeneous PTEN loss rate were 14.1% and 13.2%, respectively. Expression of PTEN protein was significantly associated with ISUP 2019 grade groups; however, there was no significant correlation between PTEN expression and other clinicopathological characteristics.

ACKNOWLEDGEMENT

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