The Rate of Phosphatase and Tensin (PTEN) Gene Expression Loss in Prostate Cancer and its Link to Tumor Upgrading

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Purpose: Recent studies have provided reliable evidence for a relationship between loss of PTEN gene expression and prognosis in patients suffering from prostate cancer, although the results have been somewhat diverse in different populations. We aimed to assess PTEN gene expression loss by immunohistochemistry in prostate cancer and also its link to tumor upgrading in a group of affected patients undergoing radical prostatectomy.

Materials and Methods: This cross-sectional study was performed on 58 tissue samples sourced from the patients with prostate cancer and undergoing radical prostatectomy. TRUS-guided needle biopsies of the cancer tissue samples with histological grade groups of I to IV (the Gleason scores of 6 to 8) were prepared as the study samples. 29 patients with Gleason score (6 to 8) whose tumors on needle biopsy upgraded to Gleason score 7, 8 or 9 at prostatectomy (cases) were compared with 29 patients with Gleason scores of 6, 7 or 8 on both biopsy and prostatectomy samples (controls). Immunohistochemistry (IHC) technique was employed to determine PTEN gene expression status.

Results: Loss of PTEN gene expression was found in 62.1% of upgraded cases compared with 27.6% of controls, indicating a statistically significant difference, revealing a meaningful association between the loss of PTEN gene expression and tumor upgrading. Furthermore, we demonstrated that deletions of PTEN gene expression and increased Gleason score in control and upgraded case groups, did not reach statistical significance.

Conclusion: A high rate of PTEN gene expression loss can be detected in prostate cancer tumor tissue, and this loss of gene expression is associated with tumor upgrading.

Keywords: prostate cancer; PTEN gene expression loss; immunohistochemistry; radical prostatectomy.

INTRODUCTION

Prostate cancer is identified as one of the most frequent fatal cancers among men in both developed and developing countries[(1)]. The variant histology PCa, according to the 2016 World Health Organization (WHO) classification of tumors of the prostate are summarized in Table1. The 2016 WHO definition is as follows: New entity 'Intraductal carcinoma of the prostate that has some features of high-grade prostatic intraepithelial neoplasia (HGPIN) but has much greater histological atypia and associated with high-grade and high-stage prostate carcinoma[(2)].

Pathological staging along with determination Gleason score is now used to predict the clinical prognosis of this carcinoma, however, due to significant clinical and prognostic heterogeneity of this tumor as well as different identified factors affecting its poor prognosis, selecting the best diagnostic, prognostic and therefore therapeutic approaches remains already challenging[(3)]. Additionally, the molecular mechanisms of prostate carcinogenesis are unclear. In this regard, the association of some genomic variants and polymorphisms with the pathogenesis of this cancer and also poorer prognosis have been recently discovered and are under assessment.

Phosphatase and Tensin (PTEN) is a tumor suppressor gene located on chromosome 10 (10q23.31) encoding a dual-specificity Phosphatase[(4)]. The inactivation of this gene or its specific polymorphism have been shown to be associated with different malignancies such as melanoma, endometrial carcinoma, lung cancer, squamous cell carcinoma, renal cell carcinoma, breast cancer, osteosarcoma and glioma[(5,6,7,8)].

The tumor suppressor activity of PTEN gene is suggested to be related to its ability to dephosphorylate

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Table 1. 2016 World Health Organization (WHO) classification of carcinomas and neuroendocrine tumors of the prostate (2)

Glandular neoplasms
Acinar adenocarcinoma
Atrophic
Pseudohyperplastic
Microcystic
Foamy gland
Mucinous (colloid)
Signet ring-like cell
Pleomorphic giant cell
Sarcomatoid
Intraductal carcinoma
Ductal adenocarcinoma
Urothelial carcinoma
Squamous neoplasms
Adenosquamous carcinoma
Squamous cell carcinoma
Basal cell carcinoma
Neuroendocrine tumors
Adenocarcinoma with neuroendocrine differentiation
Well-differentiated neuroendocrine tumor
Small-cell neuroendocrine carcinoma
Large-cell neuroendocrine carcinoma

phosphoproteins or phospholipids that can negatively regulate the activity of the phosphatidylinositol 3-kinase pathway, a powerful molecular pathway involving malignant cell proliferation and differentiation. Thus, loss of PTEN or its down-regulation may enhance tumor progression, malignant cell proliferation and tumor angiogenesis[9].

In this regard, it has been demonstrated that the over-expression of PTEN is associated with inhibiting the progression of cell cycle by G1 phase arrest, inhibiting cell migration and also inducing cellular apoptosis[10]. Within the last decade, deletion of this gene has been tracked in about 40% of patients with prostate cancer and is related to aggressive and metastatic tumors[11]. However, the rate of such mutations of the molecular behaviors of this gene in prostate cancer patients has been indicated to be divergent in different populations. In addition, the prognostic value of these mutations in predicting cancer-related poorer prognosis has not been well clarified. Hence, we aimed to assess PTEN gene loss in prostate cancer and also its link to tumor upgrading in a group of affected patients undergoing radical prostatectomy using a validated, relatively simple and inexpensive test

MATERIALS AND METHODS

This cross-sectional study was performed on a total of 58 tissue samples sourced from patients with prostate cancer and undergoing radical prostatectomy, including 29 with upgrading from the needle biopsy to prostatectomy samples and 29 with no upgrading. The study was conducted in the pathology department of Shahid Modarres Hospital in Tehran between 2018 and 2020 and was ethically approved by the ethical committee at Shahid Beheshti University of Medical Sciences. Needle biopsies of the cancer tissue samples with histological grade groups of I to IV (Gleason scores of 6 to 8) were chosen. Those with the histological grade group of V or the Gleason score of 9-10 were excluded from the study. For every case, a representative paraffin block that contained both tumor and benign prostate tissue was selected from the corresponding prostatectomy sample. 29 tumors with Gleason scores of 6 to 8 on biopsy upgraded to Gleason score 7, 8 or 9 at prostatectomy (cases) were compared with 29 tumors with Gleason scores 6, 7 or 8 on both biopsy and prostatectomy specimens (controls). All biopsies and radical prostatectomy slides were reviewed and graded according to the International Society of Urological Pathology (ISUP) grading system. A single block with the largest percentage of involvement by tumor was selected for PTEN Immunostaining. Immunostaining was performed according to the standard protocol and loss of gene expression was determined and compared between the study groups and the association of gene expression loss and tumor upgrading was assessed. In this study, the loss of PTEN gene expression was determined as lack of complete staining of the nucleus and cytoplasm in at least 10% of tumor cells[12] (**Figures.1 and 2**).

For statistical analysis, results were summarized by frequency (percentage) for categorical variables and median (interquartile range) for quantitative variables. The categorical variables were compared using the Chi-Square test. The Gleason score variable was compared between subgroups of PTEN protein loss (Negative, Positive) using Mann-Whitney U test. P values of $\leq$ 0.05 were considered statistically significant. For the

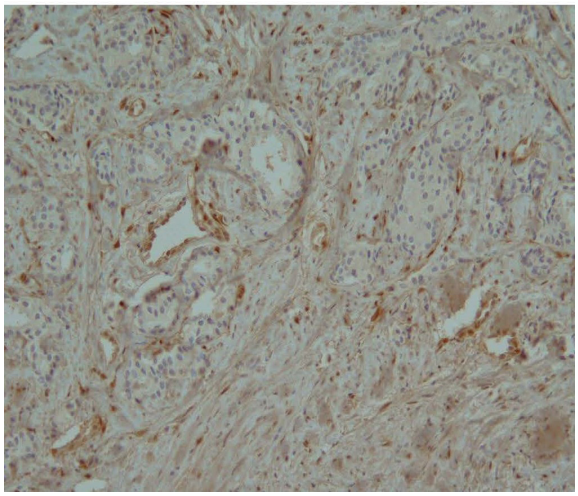

Figure 1. Needle biopsy specimen of prostatic adenocarcinoma showing PTEN-deficient, lack of complete staining of the nucleus and cytoplasm (Arrows)

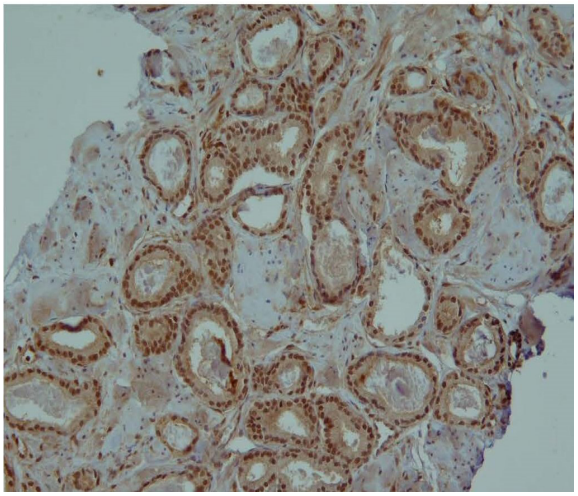

Figure 2. PTEN-positive staining of the nucleus and cytoplasm by immunohistochemistry (Arrows)

Table 2. PTEN protein loss on biopsy according to the Gleason score and Tumor grade

Group	Gleason Score	Tumor Grade	Gleason Score Frequency(N)	PTEN Loss Frequency	PTEN Loss Rate (%)
Case	Score 6	1	17	10	58.8
	Score 7	2-3	7	4	57.2
	Score 8	4	5	4	80.0
	Total		29	18	62.1
Control	Score 6	1	13	2	15.4
	Score 7	2-3	14	6	42.9
	Score 8	4	2	0	0.0
	Total		29	8	27.6

statistical analysis, the statistical software SPSS version 23.0 for windows (IBM, Armonk, New York) was used.

RESULTS

In the case and control groups, a gleason score of 6 was found in 17 and 13 samples, a gleason score of 7 in 7 and 14 samples and the gleason score of 8 in 5 and 2 samples, respectively (**Table 2**). According to the reports of IHC staining, loss of PTEN gene expression and increased Gleason score in control and upgraded case groups, did not reach statistical significance (*P*-value = 0.429, *P*-value = 0.611; **Table 3**). Furthermore, loss of PTEN gene expression was present in 62.1% of upgraded cases compared with 27.6% of controls, indicating a statistically significant difference (*P*-value = 0.008). The odds of positive PTEN protein loss of patients in the case group are significantly higher than those of patients in the control group (OR 4.29; 95% CI: 1.42-12.99; **Table 4**). In this regard, it was revealed a meaningful association between the loss of PTEN gene expression and tumor upgrading. Multivariate analysis to adjust confounders was not possible due to a lack of sample size.

DISCUSSION

Primary prostate cancer is a multifocal phenomenon; however different studies demonstrated that a large fraction of prostate cancer shows evidence of multiclonality. In this regard, attempts are mainly focusing on the identification of molecular processes and genetic diversities associated with tumor progression and metastasis. In other words, progressive and high-grade prostate cancer is now suggested to be associated with a high level of morphologic and molecular diversity that may be even linked to resistance to different therapeutic strategies[13]. Due to the close link between loss of PTEN gene expression and prostate tumor prognosis and because of the different molecular behavior of this gene in different populations, we aimed to assess the rate of loss of PTEN gene in prostate cancer patients and to assess its possible link with tumor upgrading assessed by histological studies. We revealed a prevalence rate of loss of PTEN gene expression in 62.1% of cases with tumor upgrading in subsequent prostatectomy samples, which was significantly higher than that observed in the control group (27.6%). The rate of PTEN protein loss has been very divergent in different studies. We potentially believe that the main reason for this diversity is related to the rate of tumor progression and in fact to its grade. In this context, we could show a close link between PTEN protein loss and tumor upgrading. Other studies have offered different results depending on the grade of the tumor, but almost all of them indicate a strong relationship between the grade of the tumor and the amount of gene expression loss. As shown by Liana B Guedes in 2017[14], the rate of PTEN Loss in Gleason score 7 was found to be 27.0%, however, it was significantly higher in European American men than in African American men (31% versus 9%). They could also show that the rate of gene expression loss was associated with higher tumor stage and grade at prostatectomy. Picanço-Albuquerque in 2019[15] showed PTEN deletions by FISH in 18.9% of tumors, and PTEN protein loss by IHC in 16.3% of tumors, but in both assessments, PTEN deletion was significantly associated with positive margin and Gleason score upgrading. In another study by Maisa Yoshimoto et al in 2013[16], PTEN deletion was observed in 42% of patients with prostate cancer and the deletions were significantly associated with Gleason grades 4 or 5 compared to grade 3. In an earlier study by McMenamin et al in 1999[17], 15.6% of prostate cancer were positive, 64.2% were mixed (containing both positive and negative tumor cells), and only 20.2% were negative for PTEN gene expression. They also found that the complete absence of PTEN expression correlated with an advanced pathological stage and also with the Gleason score, especially a Gleason score of 7 or higher.

Overall, due to the central role of PTEN gene expression in inhibiting malignant tumor progression, deletion of PTEN gene has a critical role in the initiation of certain tumors such as prostate cancer. In any case, inactivation and non-expression of this gene is associated with continued cell cycle of tumor cells and inhibition of apoptosis in these malignant cells. Thus, lack of expression of this inhibitory gene is associated with tumor

Table 3. Comparison of Gleason scores based on PTEN protein loss in the control and case groups

Group	PTEN Loss		*P*-value*
	Positive	Negative	
Case	6.0 (6.0-7.2)	6.0 (6.0-7.0)	0.611
Control	7.0 (6.2-7.0)	6.0 (6.0-7.0)	0.429

Data are median (Interquartile range), *Mann-Whitney test.

Table 4. Distribution of PTEN protein loss in the control and case groups

PTEN loss	Control	Case	P-value*	OR (95% CI)
Positive	8(27)	18(62)	0.008	4.29 (1.42-12.99)
Negative	21(72)	11(37)		

Data are frequency (percentage), OR: odds ratio, * chi-square test.

grade progression. Although this study did not evaluate the precise ability and value of this marker in predicting disease prognosis, it is obvious that a higher grade may be expected in cases of lack of expression of this gene with a possible high prognostic value in predicting disease outcome or results of various antitumor therapies. Of course, based on the results of various studies, it should be noted that the loss of expression of this gene can be influenced by numerous underlying factors such as demographic characteristics, techniques used to track gene expression and tumor histological features, keeping in mind that expression or loss of gene expression and its prognostic potential may be community-specific.

CONCLUSIONS

As of recent, finding a relationship between morphologic features (traditionally H&E and other histochemical stains viewed under light microscope) and molecular changes (antigen expression, gene expression, etc.) has been a goal to further enhance the detection, predict the prognosis and possibly choose the best treatment between various available modalities. While Gleason score and grade grouping is one of the most important parameters in the management of prostatic carcinoma, there is little doubt that other important factors play a role in its pathogenesis, the identification of which can help the clinician to choose the best strategy to treat, say for example two Gleason score six-carcinomas detected in needle biopsy samples, which may, however, show a different behavior from each other and require quite different treatments. In the population under our study, loss of PTEN expression was revealed in about 60% of our case group. It can be finally concluded that higher rates of PTEN expression loss can be expected in upgraded prostate cancer tumor tissue. So, the rate of PTEN protein loss is linked to the tumor upgrading, explaining the relationship between loss of expression of this gene and a worse prognosis of the disease.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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