Translational Research in Urology

Home Page: www.transresurology.com

Original Article

Phosphatase and Tensin Gene Association with Features of Aggressive Prostate Cancer

Mojtaba Saadati[1], Saeed Tamehri[2], Mohsen Pour Kamali[2], Diana Taheri[3*]

[1]*Imam Hossein University, Tehran, Iran*

[2] *Urology Research Center, Tehran University of Medical Science, Tehran, Iran*

[3] *Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran*

HIGHLIGHTS

- PTEN expression suppressed in patients with prostate cancer.
- PTEN expression had significant association with clinicopathological parameters.
- PTEN expression can be used a presictive biomarker in prostate cancer.

ARTICLE INFO

Receive Date: 09 March 2021
Accept Date: 02 April 2021
Avaliable Online: 02 April 2021
DOI: 10.22034/TRU.2021.276823.1062

***Corresponding Author**:
Diana Taheri
Email: diana1380@yahoo.com
Address: Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Hezar Jerib St., Isfahan, Iran.

ABSTRACT

Introduction
The current study examined the clinical impacts of phosphatase and tensin (PTEN) expression in prostate cancer (PCa) using immunohistochemistry.

Methods
50 patients with mean age of 66.4±7.3 years who had undergone prostatectomy surgery with the diagnosis of PCa, were enrolled in the study. We collected 50 paraffin blocks from the malignant part and 50 paraffin blocks from the healthy part of each patient's prostate. We considered malignant and healthy parts as the case and the control, respectively. Clinical and pathological information of the patients were gathered and their associations with PTEN status were assessed using odds ratios (ORs) analysis.

Results
The significant associations between tumor stage, perivascular invasion, perineural invasion, marginal involvement, extraprostatic extension, and biochemical recurrence (as assed by post-surgical prostate-specific antigen (PSA)) and PTEN expression were detected. For patients negative for PTEN, the odds ratio of the higher stage, perivascular invasion, perineural invasion, marginal involvement, and extraprostatic extension in comparison to patients positive for PTEN were estimated 7.5 (95%CI: 2.01,27.86), (95%CI: 1.65-25.57), 7.8 (95%CI:1.54-40.09), 9.78 (95%CI:2.33-41.08), and 4.84 (95%CI:1.07-21.84), respectively. Concerning biochemical recurrence, ORs was calculated 0.30 (95%CI:0.09-1.02) for PTEN positive patients compare to PTEN negative patients.

Conclusions
Since PTEN loss was associated with fe¬atures of aggressive PCa, it can be concluded that loss of PTEN would lead to more aggressive PCa and thereby, lower clinical outcomes.

Keywords: Prostate Cancer; Phosphatase; Tensin; Prostate-Specific Antigen; Biochemical Recurrence

Introduction

Among cancers in men, prostate cancer (PCa) is the leading cause of mortality rate in the United States of America (USA). Consequently, there is a great amount of tendency towards the study of PCa's etiologies (1). The wide spectrum of factors mainly including age, race, hormonal factors, diet, lifestyle, and familial history can participate in the etiology of PCa (2, 3). Additionally, several genes have a well-known role in inducing PCa and can account for tumor invasion that leads to metastasis

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International license (https://creativecommons.org/licenses/by-nc/4.0/). Noncommercial uses of the work are permitted, provided the original work is properly cited.
Copyright © 2021 Urology Research Center (URC).

and increase the odds of fatality (4). Although tremendous improvements in terms of early diagnosis of PCa have occurred a few molecular markers that can differ lethal disease from indolent one, are exist (5). Phosphatase and tension (PTEN) are tumor suppressor genes that are located on the chromosome of 10q23.3, and It has been proven that PTEN is part of the signal transduction pathways involved in cell growth, proliferation, apoptosis as well as cell cycle control. Mutation and loss of PTEN gene can contribute to interference in some crucial pathways which play pivotal roles in the initiation of cancers (6, 7). PTEN not only promotes the emergence of tumor cells but has a close association with a patient's survival and prognosis as well (8-10). Some works highlighted PTEN loos has a deleterious effect on the health of patients with PC through shortening time to metastasis, advancing disease's stages, decreasing time to disease recurrence, more resistance to hormone therapy, and the higher chance of invasion to extraprostatic tissues (17–19). We designed a study to evaluate the correlation of PTEN expression, which was assessed by immunohistochemistry staining, with features of aggressive PCa.

Methods

This was a cohort retrospective study that investigated 50 patients who had undergone radical prostatectomy with the diagnosis of prostate adenocarcinoma between 2019-2020 in Sina Hospital. In this cohort, radical prostatectomy was conducted based on the procedure explained by Walsh et al (11). In this study, we collected 50 paraffin blocks from the malignant part and 50 paraffin blocks from the healthy part of each patient's prostate. We considered malignant and healthy parts as the case and the control, respectively. Initial patients' information including age, smoking status, grade of tumor, stage of the tumor, Gleason score, perivascular and perineural invasion, and marginal involvement were extracted from patients' records and pathological reports. For assessing biochemical recurrence, we measured serum PSA values 1 month following radical prostatectomy and as cited above, serum values higher and lower than 0.2 ng. ml was considered as positive and negative, respectively. The samples of the prostate were evaluated twice by an expert pathologist. All PCa were adenocarcinoma, acinar type, and other types of PCa, and PCa with stage 1 and 4 were excluded from the current study. For immunohistochemistry (IHC) test and PTEN expression in the samples, first, the selected blocks were cuts to 3 microns' thickness and incubated for at least 12 hours in the incubator or for 20-22 minutes at 58-62 ° C and then, the tissues were placed in xylenol lol solution for paraffin removal. The alcohols were poured with 100% -100% -96% and 70% aliquots to be disinfected and washed with running water. They were then washed with phosphate buffer solution (PBS) and after that, immersed in 10% oxygen for 10 min under dark conditions. They re-wash with phosphate buffer solution and transfer to the citrate-containing container at pH = 6. Then, they were placed in the autoclave at 120 ° C for 30 minutes at a pressure of 1.5 bar, the slides were cooled after removal autoclave and tissue area were determined by IHC pen. Then, without washing, add anti-PTEN antibody and incubate for 60 minutes and then, they were washed twice with phosphate-buffered saline 2 times and at any time for 5 min and the secondary antibody (Envision solution) was added and incubated for 30 min, again they were washed twice with phosphate-buffered saline for 5 min, Chromogen and DAB (1ccDAB per 50 la) were added and incubated for 10 min, Rinse with running water to remove excess dyes for 1 minute and then hematoxylin dye was added and samples were washed with that. Of note, the anti-PTEN Antibody kits used for this project are from the brand of Master diagnosis (Spain) with Lot number 05390005. The detection kit, which includes a chromogen – DAB and secondary antibody from the DBS brand (US) with Lot number J787 and also, the slides were silane-coated (Cytoglass, China). The intensity of staining was graded on a scale of + 0-3. While 0 means no staining, +1 very poor or minor cell staining, +2 good or most staining, and +3 total cytoplasm staining in most cells and High intensity. We considered 0 and +1 as negative, and +2 and +3 as positive for PTEN.

Statistical analysis

The continuous variables are reported using mean (standard variation), and the discrete ones as number (percent). Moreover, the chi-square and odds ratio tests were used for analyzing categorical data.

Results

The mean age of patients was 66.4±7.3 years. Both groups of patients were age-matched (p-value=0.242). Moreover, both groups were similar for smoking status (p-value=0.377). PTEN was negative in 20 out of 50 patients. PTEN was negative in 20 (40%) and 2 (4%) of the samples of the case and control group, respectively. There was a significant difference between the case and the control group in terms of PTEN expression (p-value <0.001). Among PTEN negative patients, eight patients had stage 2 and 12 patients had stage 3 PCa. Among PTEN positive patients, 25 patients had stage 2 and five patients had stage 3 PCa. There was a significant difference between PTEN positive and negative patients in terms of stage of the tumor (p-value=0.002). We also found out that PTEN negative patients are at 7.5 (95%CI: 2.01-27.86) risk of having stage 3 than stage 2 of PCa. Perivascular and perineural invasion were found in 10 and 18 PTEN negative patients, respectively. For positive

PTEN patients, perivascular and perineural invasion was detected in 4 and 16 patients, respectively. Significant differences between PTEN positive and negative patients were appeared (p-value =0.009 and 0.012, respectively). The odds ratio for the perivascular and perineural invasion were measured 6.5 (95%CI: 1.65-25.57) and 7 (95%CI:1.59 -30.79), respectively.

The extraprostatic extension was detected in seven and three PTEN negative and positive patients, respectively and the difference was marginally significant (p-value= 0.067) (odds ratio 2.07 [95%CI:1.07-4.02]). Marginal involvement was seen in 17 and 11 PTEN negative and positive patients, respectively, which was significantly different (p-value=0.001), and the odds ratio for marginal involvement was estimated 6.33 (95%CI: 1.87-21.40).

Half of the patients with positivity for PTEN had post-surgical PSA higher than 0.2 ng/ml compared with 23% of PTEN negative patients (p-value=0.050). The odds ratio of having post-surgical PSA higher than 0.2 ng/ml in PTEN positive patients is 0.30 (95%CI:0.09-1.02) compared with PTEN negative patients.

Information on the Gleason score is presented in Table 1. A significant difference between the two groups for Gleason score was found.

Of six factors that had significant associations with PTEN expression, 10 patients had none of the factors and six patients had all of them (Table 2).

Of 20 patients who were negative for PTEN expression, all of them had at least one factor and of 30 patients who were positive for PTEN expression, 10 had none of the investigated factors and 20 had at least one factor.

Discussion

Our study investigated the association between PTEN expression and patients', tumor, and histopathological characteristics including age, smoking status, stage, grade, Gleason score, extraprostatic extension, marginal involvement, perivascular invasion, perineuralinvasion, and biochemical recurrence, which assessed by post-surgical PSA.

We demonstrated that there are significant associations between PTEN expression and perivascular invasion, perineural invasion, extraprostatic extension, marginal involvement, stage, and biochemical recurrence. PTEN deletion or mutation is implicated in a variety of malignancies in particular PCa. Alteration in the PTEN gene is known as the most frequent loss tumor suppressor gene in PCa and has different incidence in different studies (12-14). Lines of clinical research have been conducted to assess the association between PTEN status and factors that enhance the aggressivity of the tumor (15). The bulk of evidence claimed that the incidence of PTEN loss or mutation increases as the tumor progress (16, 17). Moreover, an experimental study has shown that PTEN loss can initiate prostate tumorigenesis (18). To sum up, the PTEN gene maintains a mandatory role not only in Pca progression but also in PCa initiation.

According to the American Urological Association and The European Association of Urology, biochemical recurrence in PCa is defined as the prostate-specific antigen (PSA) higher than 0.2 ng/ml after radical prostatectomy and is taken into account as the outcome (19). The bulk of evidence expresses that biochemical recurrence is associated with an increased risk of tumor progression and metastasis (19). In the current study, we found out that the absence of PTEN expression led to higher biochemical recurrence rates in PCa. Similarly, Chaux et al., demonstrated that there is a negative association between PTEN expression and biochemical recurrence following radical prostatectomy in localized PCa (20). In contrast, Bedolla et al., claimed that PTEN alone cannot predict PCa biochemical recurrence; nevertheless, in combination with Akt can be considered as a suitable predictor of biochemical recurrence (21). Evidences are in support of the fact that perineural invasion (22) and perivascular invasion (23) associated with worsen clinical

Table 1. Gleason score of enrolled patients based on PTEN status

Gleason score	PTEN positive	PTEN negative	Total
3+3	1	2	3
3+4/4+3	3	8	11
4+4/3+5	4	10	14
4+5	5	5	10
5+5	7	5	12
Total	20	30	50

Table 2. Distribution of enrolled patients based on risk factors

Risk factors	Patients (Frequency)	Patients (Percent)
0	10	20.00
1	13	26.00
2	6	12.00
3	4	8.00
4	8	16.00
5	3	6.00
6	6	12.00
Total	50	100.00

outcomes in PCa patients. We clarified that the chance of perineural and perivascular invasion in PCa patients who are negative for PTEN expression versus positive for PTEN expression is much higher. Olar et al., did not find any significant association between perineural invasion diameter and PTEN expression in 640 specimens of radical prostatectomy (24). Yoshimoto et al., showed that in PCa patients, PTEN genomic deletion is associated with perineural invasion with hazard ratio of 6.22 (95%CI: 1.51-25.60) (17). As far as we are aware, no study by far evaluated the association between perivascular invasion and PTEN expression in PCa patients who experienced radical prostatectomy.

A meta-analysis pointed out that marginal involvement increases the risk of recurrence-free survival, overall survival, and overall mortality following radical prostatectomy (25). In the present study, positive marginal status was expected to be found significantly higher in PTEN negative patients compared with PTEN positive patients. Likewise, in a large cohort of 13,665 PCa patients in Germany, positive marginal status was significantly associated with loss of PTEN gene with hazard ratio of 1.2 (95%CI:1.03-1.32) for PTEN loss (26). It has been postulated that more unfavorable outcomes can be detected in PCa patients with extraprostatic extension versus PCa patients without extraprostatic extension (27). We illustrated that PTEN loss is associated with an increased risk of extraprostatic extension in PCa patients. A study examined the relationship between PTEN expression, which was assessed using fluorescence in situ hybridization, and several clinical outcomes including the extraprostatic extension in 107 consecutive PCa patients. Extraprostatic extension appeared to be significantly associated with PTEN genomic deletion with a hazard ratio of 3.2 (95%CI: 1.72-5.94) (17).

It has been postulated that patients with a higher clinical stage of PCa are at higher risk of being positive for factors associated with higher mortality rate including positive margin status and extracapsular extension (28, 29). In the current study, we enrolled patients with stage 2 or 3 of PCa. We compared the PTEN expression of PCa patients with stage 2 and stage 3 and found out that patients with stage 3 of PCa are more tended to be negative for PTEN than patients with stage 2 of PCa. In line with our finding, several studies declared higher stages of PCa can be expected as the expression of PTEN decreases (30-32). In the study of Lotan et al, the significant association between PCa stage and PTEN expression was reached and it was shown that patients with stage 3b of PCa had a hazard ratio of 1.5 (95% CI: 1.28,1.69) in comparison to patients with stage 3a of PCa and patients with stage 4 of PCa had a hazard ratio of 1.3 (95% CI: 0.91, 1.76) in comparison to patients with stage 3b of PCa (26).

Conclusions

In the present cohort, we assessed the correlation between PTEN expression and features of aggressive PCa. We demonstrated that PTEN loss would lead to a higher tumor clinical-stage, positive margin status, extraprostatic extension, perivascular invasion, perineural invasion, and biochemical recurrence. Since the mentioned factors are postulated to be associated with poor prognosis in PCa patients, it can be concluded that PTEN loss correlates with worsen clinical outcomes.

Authors' contributions

All authors contributed equally.

Acknowledgments

Special thanks to Urology Research Center (URC), Tehran University of Medical Sciences (TUMS).

Conflict of interest

All authors declare that there is not any kind of conflict of interest.

Funding

There was no founding.

Ethics statement

The study was done under the Tehran University of Medical Science ethical committee (IR.TUMS.MEDICINE.REC.1399.986).

Data availability

Data will be provided by the corresponding author on request.

Abbreviations

PCa	Prostate cancer
PTEN	Phosphatase and tensin
PSA	Prostate-specific antigen
ORs	Odds ratio

References

1. Pernar CH, Ebot EM, Wilson KM, Mucci LA. The epidemiology of prostate cancer. Cold Spring Harbor perspectives in medicine. 2018;8(12):a030361.
2. Gann PH. Risk factors for prostate cancer. Reviews in urology. 2002;4(Suppl 5):S3.
3. Patel AR, Klein EA. Risk factors for prostate cancer. Nature Clinical Practice Urology. 2009;6(2):87-95.
4. Perdana NR, Mochtar CA, Umbas R, Hamid A. The risk factors of prostate cancer and its prevention: a literature review. Acta Med Indones. 2016;48(3):228-38.
5. Shen MM, Abate-Shen C. Molecular genetics of prostate cancer: new prospects for old challenges. Genes & development. 2010;24(18):1967-2000.
6. Chow LM, Baker SJ. PTEN function in normal and neoplastic growth. Cancer letters. 2006;241(2):184-96.
7. Worby CA, Dixon JE. Pten. Annual review of biochemistry. 2014;83:641-69.
8. Que W-c, Qiu H-q, Cheng Y, Liu M-b, Wu C-y. PTEN in kidney cancer: A review and meta-analysis. Clinica chimica acta. 2018;480:92-8.
9. Wise HM, Hermida MA, Leslie NR. Prostate cancer, PI3K, PTEN and prognosis. Clinical science. 2017;131(3):197-210.
10. Xiao J, Hu C-P, He B-X, Chen X, Lu X-X, Xie M-X, et al. PTEN expression is a prognostic marker for patients with non-small cell lung cancer: a systematic review and meta-analysis of the literature. Oncotarget. 2016;7(36):57832.
11. Walsh PC, editor Radical prostatectomy: a procedure in evolution. Seminars in oncology; 1994.
12. Abeshouse A, Ahn J, Akbani R, Ally A, Amin S, Andry CD, et al. The molecular taxonomy of primary prostate cancer. Cell. 2015;163(4):1011-25.
13. Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat J-P, et al. Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. Nature genetics. 2012;44(6):685-9.
14. Berger MF, Lawrence MS, Demichelis F, Drier Y, Cibulskis K, Sivachenko AY, et al. The genomic complexity of primary human prostate cancer. Nature. 2011;470(7333):214-20.
15. Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, et al. Clinical implications of PTEN loss in prostate cancer. Nature Reviews Urology. 2018;15(4):222.
16. Troyer DA, Jamaspishvili T, Wei W, Feng Z, Good J, Hawley S, et al. A multicenter study shows PTEN deletion is strongly associated with seminal vesicle involvement and extracapsular extension in localized prostate cancer. The Prostate. 2015;75(11):1206-15.
17. Yoshimoto M, Cunha I, Coudry R, Fonseca F, Torres C, Soares F, et al. FISH analysis of 107 prostate cancers shows that PTEN genomic deletion is associated with poor clinical outcome. British journal of cancer. 2007;97(5):678-85.
18. Poliseno L, Salmena L, Riccardi L, Fornari A, Song MS, Hobbs RM, et al. Identification of the miR-106b~ 25 microRNA cluster as a proto-oncogenic PTEN-targeting intron that cooperates with its host gene MCM7 in transformation. Science signaling. 2010;3(117):ra29-ra.
19. Van den Broeck T, van den Bergh RC, Briers E, Cornford P, Cumberbatch M, Tilki D, et al. Biochemical recurrence in prostate cancer: The European association of urology prostate cancer guidelines panel recommendations. European urology focus. 2020;6(2):231-4.
20. Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, et al. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. Modern pathology. 2012;25(11):1543-9.
21. Bedolla R, Prihoda TJ, Kreisberg JI, Malik SN, Krishnegowda NK, Troyer DA, et al. Determining risk of biochemical recurrence in prostate cancer by immunohistochemical detection of PTEN expression and Akt activation. Clinical cancer research. 2007;13(13):3860-7.
22. Ahmad AS, Parameshwaran V, Beltran L, Fisher G, North BV, Greenberg D, et al. Should reporting of peri-neural invasion and extra prostatic extension be mandatory in prostate cancer biopsies? correlation with outcome in biopsy cases treated conservatively. Oncotarget. 2018;9(29):20555.
23. Herman CM, Wilcox GE, Kattan MW, Scardino PT, Wheeler TM. Lymphovascular invasion as a predictor of disease progression in prostate cancer. The American journal of surgical pathology. 2000;24(6):859-63.
24. Olar A, He D, Florentin D, Ding Y, Wheeler T, Ayala G. Biological correlates of prostate cancer perineural invasion diameter. Human pathology. 2014;45(7):1365-9.
25. Zhang L, Wu B, Zha Z, Zhao H, Yuan J, Jiang Y, et al. Surgical margin status and its impact on prostate cancer prognosis after radical prostatectomy: a meta-analysis. World journal of urology. 2018;36(11):1803-15.
26. Lotan TL, Heumann A, Rico SD, Hicks J, Lecksell K, Koop C, et al. PTEN loss detection in prostate cancer: comparison of PTEN immunohistochemistry and PTEN FISH in a large retrospective prostatectomy cohort. Oncotarget. 2017;8(39):65566.
27. Danneman D, Wiklund F, Wiklund NP, Egevad L. Prognostic significance of histopathological features of extraprostatic extension of prostate cancer. Histopathology. 2013;63(4):580-9.
28. Buhmeida A, Pyrhönen S, Laato M, Collan Y. Prognostic factors in prostate cancer. Diagnostic pathology. 2006;1(1):1-15.
29. Freedland SJ, Aronson WJ, Presti Jr JC, Amling CL, Terris MK, Trock B, et al. Predictors of prostate-specific antigen progression among men with seminal vesicle invasion at the time of radical prostatectomy. Cancer: Interdisciplinary International Journal of the American Cancer Society. 2004;100(8):1633-8.
30. Koksal IT, Dirice E, Yasar D, Sanlioglu AD, Ciftcioglu A, Gulkesen KH, et al., editors. The assessment of PTEN tumor suppressor gene in combination with Gleason scoring and serum PSA to evaluate progression of prostate carcinoma. Urologic Oncology: Seminars and Original Investigations; 2004: Elsevier.
31. McMenamin ME, Soung P, Perera S, Kaplan I, Loda M, Sellers WR. Loss of PTEN expression in paraffin-embedded primary prostate cancer correlates with high Gleason score and advanced stage. Cancer research. 1999;59(17):4291-6.
32. Schmitz M, Grignard G, Margue C, Dippel W, Capesius C, Mossong J, et al. Complete loss of PTEN expression as a possible early prognostic marker for prostate cancer metastasis. International journal of cancer. 2007;120(6):1284-92.

Author (s) biosketches

Saadati M, Associate Professore**,** Imam Hossein University, Tehran, Iran.
Email: saadati1-m@yahoo.com

Tamehri S, MD, Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Email: tamehrysaeed@gmail.com

Pour Kamali M, MD**,** Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran.
Email: m-pourkamali@yahoo.com

Taheri D, Professore, Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.
Email: diana1380@yahoo.com

How to cite this article

Saadati M, Tamehri S, Pour Kamali M, Taheri D. Phosphatase and Tensin Gene Association with Features of Aggressive Prostate Cancer. Translational Research in Urology. 2021 Apr;3(1):32-37.

DOI: 10.22034/TRU.2021.276823.1062

URL: http://www.transresurology.com/article_128689.html