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Research Article

IMMUNOHISTOCHEMICAL ASSESSMENT OF PTEN IN DIFFERENT GRADES OF CARCINOMA PROSTATE

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A R T I C L E I N F O

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PTEN, Gleason grade, P13K-AKT pathway, Immunohistochemistry, Gleason score.

A B S T R A C T

Background: PTEN tumor suppressor gene are among most common genomic aberration in prostate cancer. Loss of which leads to activation of P13K-AKT pathway and is strongly associated with increasing gleason grade and adverse oncological outcomes.
Aim: To assess PTEN loss in different gleason grades of prostate carcinoma.
Material and methods: A total of 72 cases of prostatic carcinoma were included in the study. All cases were assessed and categorized into different grade groups. PTEN expression was assessed by immunohistochemistry (IHC) and correlated with Gleason score and grade group.
Results: PTEN expression was assessed in different Gleason score and Grade group. In Gleason score 6 (Grade group I), all the cases were positive for PTEN expression. In Gleason score 7, 75% cases were positive in Grade group II (3+4) and 15% cases were positive in Grade group III (4+3). In Gleason score 8(Grade group IV) and score 9 (Grade group V) 18.75% cases were positive. In Gleason score 10(Grade group V) all cases were negative for PTEN expression.
Conclusion: Assessment of PTEN expression loss may play a significant role in current risk stratification of prostatic carcinoma particularly in low and intermediate risk groups.

INTRODUCTION

Prostate adenocarcinoma is the second most frequent malignancy in men worldwide, (first is lung cancer). The incidence and mortality of prostate cancer worldwide correlate with increasing age, and the average age of diagnosis being 66 years. The etiology of prostate cancer are advanced age, ethnicity, genetic factors, and family history. Other factors positively associated with prostate cancer include diet (increased consumption of red meat, lower intake of fruits, vegetables, vitamins, and coffee), obesity and physical inactivity, inflammation, hyperglycemia, infection and environmental exposure to chemicals and ionizing radiation.[1] Predisposing genetic risk factor include the expression of MYC, gene. Loss of function mutation in BReast CAncer gene 2 (BRCA2) and DNA mismatch repair gene. Acquired genetic and epigenetic alteration include chromosomal rearrangement that creates TMPRSS-ETS fusion gene, silencing of p27 gene, deletion of PTEN. Deletion of PTEN is associated with accelerated cell growth and resistance to anti androgen therapy.[2]

Prostatic adenocarcinoma is an epithelial malignancy which is characterized by marked histological and clinical heterogeneity. The most common variety of adenocarcinoma is acinar type. Prostate cancer is mostly multifocal at the time of presentation and the morphological appearance of these foci can be highly variable within prostate gland and different Gleason Grades may be present within different foci.[3]

Despite the improvement in early detection of prostate cancer using Prostate Specific Antigen (PSA) and needle biopsy, the main challenge is to determine whether the cancer is clinically significant, which is likely to progress locally or metastasize. To enhance the ability to detect the tumor behavior a number of immunohistochemical markers can be used, one such immunohistochemical marker is Phosphatase and tensin homolog (PTEN)[4]

PTEN is a tumor suppressor gene located in chromosome 10q23. Germline mutation are found in Cowden syndrome.[5] PTEN is somatically mutated or deleted in many cancers including primary glioblastoma, carcinoma prostate, endometrium and melanoma.

Studies over last decade have established that loss of PTEN tumor suppressor gene is one of the most common somatic genetic aberrations in prostate cancer and is frequently associated with high risk disease. This study was done to evaluate the loss of PTEN in different grades of prostate carcinoma.

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MATERIAL AND METHOD

This observational study was conducted on 72 cases of prostatic carcinoma specimens in the Department of Pathology in collaboration with the Department of Urology at Pt. B.D. Sharma, PGIMS, Rohtak.

Inclusion criteria

Trans urethral resection of prostate (TURP), needle biopsy and radical prostatectomy having prostatic adenocarcinoma

Exclusion criteria

1. Inadequate biopsies and poorly preserved prostatic specimens
2. Tumors other than adenocarcinoma

METHODOLOGY

All the prostatic specimens were subjected to a careful and detailed gross examination. The weight, size, of TURP chips were noted.

Hematoxylin and eosin staining for routine paraffin sections was carried out as per the standard procedure.[6] All the cases were graded as per the new Gleason grading system of carcinoma prostate. Immunohistochemical staining was performed on microscopically selected samples for characteristic areas of tumor, based on standard H&E staining

Interpretation of results

Tumors grading was done as per new gleason grading system. Interpretation of immunohistochemical stains in each case was performed by subjectively by estimation of the staining index, which is stratified from (0-9) scores, and proportion of immunopositive cells. PTEN expression was determined by multiplying proportion of stained tumor cells and staining intensity

The intensity and pattern of staining was evaluated and categorized as follows:-[7]

PTEN expression was seen as brown cytoplasmic staining in tumor cells.

The staining intensity of malignant cells were stratified as :-
Score 0 (no staining)
Score 1 (mild staining)
Score 2 (moderate staining)
Score 3 (obvious staining)

Proportion of immunopositive tumor cells were calculated as: -

Percentage	Score
< 10% of tumor cells	1
10-50% of tumor cells	2
>50% of tumor cells	3

Staining interpretation

PTEN expression was determined by multiplying proportion of stained tumor cells and staining intensity and interpreted as:-

- Negative: 0-1
- Weak positive :2-3
- Moderately positive: 4-5
- Strong positive: 6-9

Control: Positive and negative control were run with each batch.

Positive: Internal control:- Brown cytoplasmic and nuclear staining in benign prostatic glands and stroma

Negative: Negative control was obtained by substituting the primary antibody with antibody of nonspecific reference.

IHC scoring of PTEN was assessed by two pathologists independently to avoid interobserver variability;

Discrepancies were solved by examining problematic cases together under microscope.

Statistical Analysis

The collected data was categorized and coded as appropriate. The data was then compiled, tabulated, and analyzed using SPSS version 24.0 software. All the data was enlisted investigation proforma (name, age, sex, CR no, clinical diagnosis and history) was collected. Association was tested using Pearson X 2 and Fishers exact tests. P Value less than 0.05 was accepted as statistically significant.

Biomedical Waste Disposal

All the biomedical waste generated during the study was discarded as per the Biomedical Waste Management and handling rules 2018.[8]

RESULTS

We included 72 cases of prostate adenocarcinoma in our study, The patient's age ranged from 47-90 years with mean age of 69.51 years and the median age was 70 years. Maximum number (31%) of the cases were in the age group of 61-70 years (fig 1). Serum PSA level was available for 52 cases. out of which. 96% had serum PSA levels >20 ng/ml This serum PSA level, produced by the secretory epithelium of the prostate gland, usually lies between 1.0 and 1.5ng/ml and a level of >4ng/ml is usually observed in >80% of carcinoma cases. In our study, majority of the cases had more than 100-fold rise in serum PSA levels. Twenty-eight percent cases had Gleason score 7 (4+3) and 11% cases had a score of 7(3+4) followed by equal proportion of cases with Gleason score scores 8 and 9 i.e., (22.0%) and 4% cases had Gleason score 10. 27.8% of cases were found to be of Gleason Grade III, 26.4% cases of GG V and 22.2% cases of GG IV followed by 12.5% and. 11.1% cases of GG I and II respectively

Table 1 Showing correlation of PTEN expression with gleason score 7(3+4) and 7(4+3)

Gleason Grade Group	No of Cases	PTEN Expression		P Value
		Positive	Negative	
II (3+4)	8	6 (75%)	2 (25%)	0.002133
III (4+3)	20	3 (15%)	17 (85%)	

Table 2 Showing correlation of PTEN expression with gleason score

gleason score	total number of cases	PTEN expression		p value
		Negative	Positive	
6	9	0 (0%)	9 (37.5%)	
7 (3+4)	8	2 (4.1%)	6 (25%)	
7 (4+3)	20	17 (35.4%)	3 (12.5%)	
8	16	13 (27.0%)	3 12.5%)	<0.001
9	16	13 (27.0%)	3 (12.5%)	
10	3	3 (6.2%)	0 (0%)	
Total cases	**72 (100%)**	**48 (66.7%)**	**24 (33.3%)**	

Table 3 Showing distribution of prostate carcinoma cases based on PTEN expression across different grade group (n=72)

Gleason Grade	Total Cases	PTEN Expression		P Value
		Positive	Negative	
GG I	9 (12.5%)	9 (100%)	0 (0%)	
GG II	8 (11.1%)	6 (75%)	2 (25%)	
GG III	20 (27.8%)	3 (15%)	17 (85%)	<0.001
GG IV	16 (22.2%)	3 (18.7%)	13 (81.3%)	
GG V	19 (26.4%)	3 (15.8%)	16 (84.2%)	

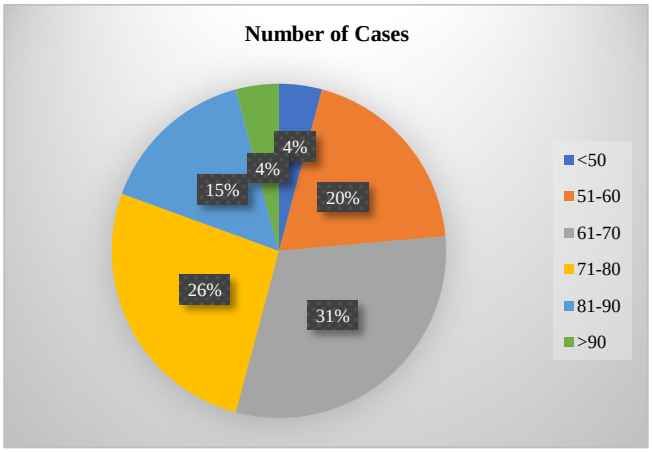

Figure 1 Distribution of prostate carcinoma cases based on age group(n=72)

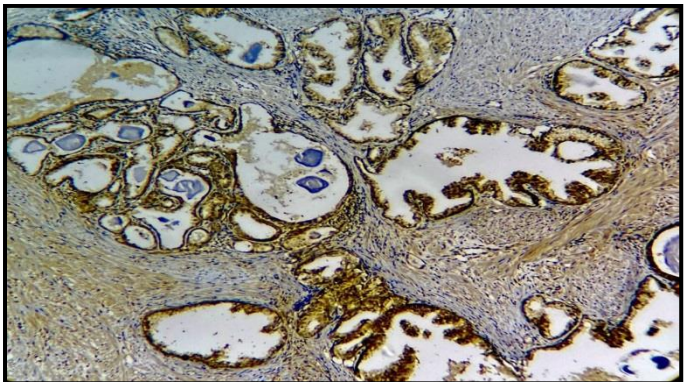

Figure 2 Positive staining of PTEN in Gleason score 3+3=6 (IHC x100)

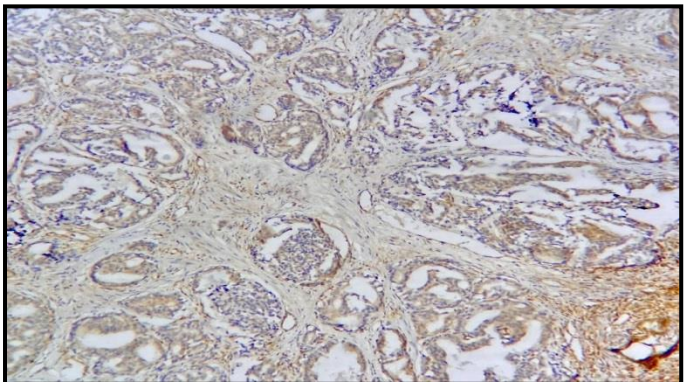

Figure 3 Positive staining for PTEN in Gleason score 3+4=7(IHC x100)

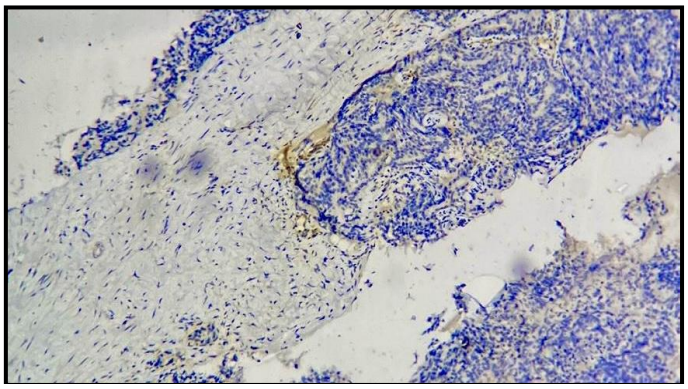

Figure 4 Negative staining for PTEN in Gleason score 4+3=7(IHC x100)

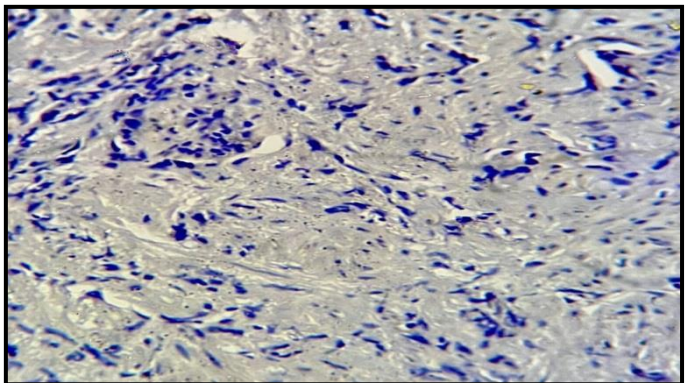

Figure 5 Negative staining for PTEN in Gleason score 4+4=8(IHC x100)

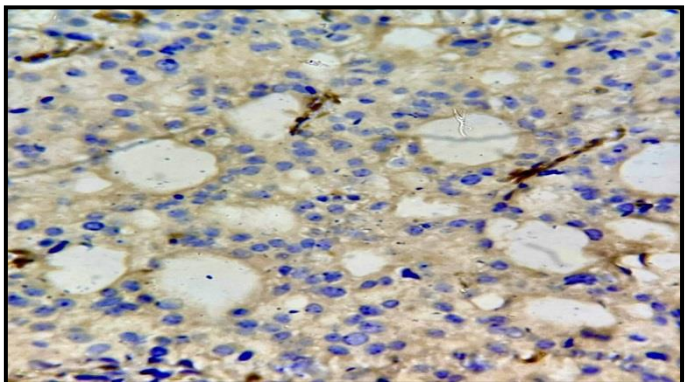

Figure 6 Negative staining for PTEN in Gleason score 5+4=9(IHC x100)

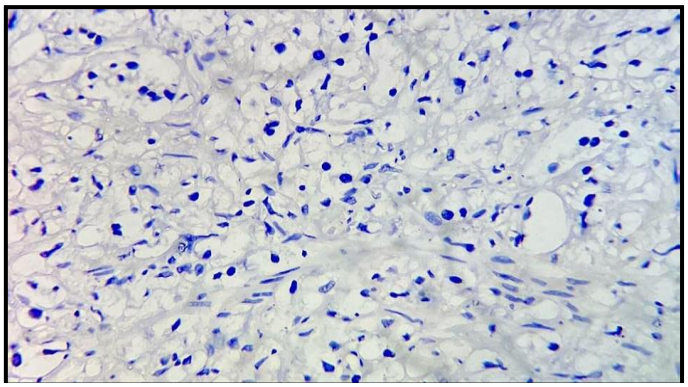

Figure 7 Negative staining for PTEN in Gleason score 5+5=10(IHC x100)

PTEN expression was assessed independently by two observers based on the previously validated scoring system[7]. According to observer 1, the total numbers of positive cases were 26 (36.1%) and negative were 46 (63.9%). Out of positive cases, 10 (13.9%) were weakly positive, 02 (2.8%) moderately positive and 14 (19.4%) were strongly positive. According to observer 2 the total numbers of negative cases were 48 (66.6%) and positive cases were 24 (33.3%). Out of

which 07 (9.8%) were weakly positive, 04 (5.5%) were moderately positive and 13 (18.1%) were strongly positive.

PTEN expression was correlated with age of the patients it was observed that with increasing age of the patient's loss of PTEN expression was more. However, these findings are statistically non-significant PTEN expression was correlated with serum PSA levels 96% had serum PSA levels >20 ng/ml and it was observed that with increasing levels of PSA, cases with loss of PTEN expression was increased but these findings were statistically non-significant.

PTEN expression was correlated with Gleason score and it was found that All of the cases with Gleason score 6 were PTEN positive. Whereas, 84% of the cases with score 9 and 10 were negative for PTEN, with increasing Gleason score there was significant decrease in positive PTEN expression (Table 2). Upon correlating PTEN expression with Gleason score 7(3+4) and (4+3), 25% of cases with Gleason score7(3+4) were negative for PTEN while 85% of cases were negative for PTEN in Gleason score 7(4+3) (Table 1). These findings were statistically significant.

PTEN expression was correlated with Gleason Grade, none of the grade group I cases had negative PTEN expression. In Gleason Grade II, 25% of cases were negative for PTEN, while 17 out of 20 cases with Gleason Grade III i.e., 85.0% had a negative PTEN expression. eighty one percent of grade IV and Eighty four percent of grade V case had negative PTEN expression (Table 3). It is clearly evident that PTEN deletion increases as the grade increases from GG I to GG V particularly in GG II and GG III, PTEN was observed to be higher in Gleason Grade III. This signifies the use of PTEN IHC as a biomarker for improved stratification of prostate adenocarcinoma with intermediate risk particularly in Gleason Grade II and III. These findings are statistically significant.

DISCUSSION

The grade and stage are the most important prognostic factors in prostate cancer grading is done using gleason system, which stratifies prostate cancer into five grades based on glandular patterns of growth. Grade 1 corresponds to well differentiated tumors in which the neoplastic glands are uniform and round in appearance and are packed into well circumscribed nodules, in contrast, grade 5 tumors do not form glands within tumor.

Pten Expression and Age of Patients

In the present study, it was observed that with increasing age of the patients loss of PTEN expression was more. However, these findings are statistically non-significant, with p value 0.56. Switlyk *et al.*[9] in their study also found non-significant correlation between age and PTEN expression.

Pten Expression and Serum PSA Levels

In our study majority of the cases i.e. 96% had serum PSA levels >20 ng/ml and it was observed that with increasing levels of PSA, cases with loss of PTEN expression was increased but these findings were statistically non-significant. In the study by Kammuna *et al.* [7] 40% of the cases had serum PSA levels >20ng/ml. In another study conducted by Cuzick *et al.*[17] for determining the prognostic value of PTEN loss among men with conservatively managed localised prostate cancer, it was found that PTEN loss was strongly correlated with baseline serum PSA of >10ng/ml with p value of 0.001. Hence, serum PSA levels screening should be routinely practiced.

Pten Expression and Gleason Score

In our study, PTEN expression was correlated with Gleason score and it was found that All of the cases with Gleason score 6 were PTEN positive. Whereas, 84% of the cases with score 9 and 10 were negative for PTEN, with increasing Gleason score there was significant decrease in positive PTEN expression. Upon correlating PTEN expression with Gleason score 7(3+4) and (4+3), 25% of cases with Gleason score7(3+4) were negative for PTEN while 85% of cases were negative for PTEN in Gleason score 7(4+3). These findings were statistically significant with P<0. 001.

Study conducted by Albuquerque *et al.* [10] it was found that 16.3% of patients had PTEN loss where out of all tumors with Gleason score 7 [GG2 and GG3], 19 (67.8%) showed PTEN loss. In another study by Lotan *et al.* [11] the PTEN loss was found in 51.0% of Gleason score7 tumors. These findings show that PTEN loss is harbored more in tumors with Gleason score 7 (4+3). Similar observation were made in several other studies which show significant association between PTEN loss and higher Gleason score along with other invasive features explored during surgery.[12,13,14]

In our study PTEN expression was correlated with Gleason score group of <7, 7, >7. There were none PTEN negative cases in score <7, 67% of cases were negative for PTEN in score 7 and 82% cases were negative for PTEN in score > 7. This finding shows a significant increase in PTEN negative cases as the score group changes from <7 to >7. These findings were statistically significant with p<0.001. In the study by Kammuna *et al.*[7] 50% of cases were PTEN negative with Gleason score of <7 and 37% cases were negative in Gleason score 7 and 60% cases were negative for PTEN with score >7. PTEN loss in Gleason score 7 may be heterogenous, further analysis to be carried out in large cohorts using a combination of FISH, IHC and digital imaging in identifying those patients who more likely to have worse prognosis.

Pten Expression and Gleason Grading

In the present study PTEN expression was correlated with Gleason Grade, none of the grade group I cases had negative PTEN expression. In Gleason Grade II, 25% of cases were negative for PTEN, while 17 out of 20 cases with Gleason Grade III i.e., 85.0% had a negative PTEN expression. eighty-one percent of grade IV and Eighty-four percent of grade V case had negative PTEN expression. It is clearly evident that PTEN deletion increases as the grade increases from GG I to GG V particularly in GG II and GG III, PTEN was observed to be higher in Gleason Grade III. This signifies the use of PTEN IHC as a biomarker for improved stratification of prostate adenocarcinoma with intermediate risk particularly in Gleason Grade II and III. These findings are statistically significant with p<0.001.

In the study conducted by Shah MD *et al.*[15] it was found that highest frequency of PTEN loss was among tumors with Gleason Grade 5 i.e., 64%, followed by GG3 i.e., 51.0%. Furthermore, in a study conducted by Hamid AA *et al.*[16] it was observed on univariate analysis that tumor PTEN expression in the lowest quartile had a significantly higher risk of developing metastatic disease in comparison to higher tumour PTEN expression i.e., HR = 1.92, 95% CI [1.02- 3.63]

We observed a statistically significant correlation of PTEN expression with Gleason score and Gleason Grade group. Loss of PTEN expression was seen associated with higher Gleason score and grade group. These results also indicate that evaluating the extent of PTEN loss allow better separation of favorable and unfavorable prognosis, particularly in Gleason score 7 and between grade II and III. Hence, loss of PTEN can be used as a biomarker for stratification of prostate carcinoma with intermediate risk.

CONCLUSION

PTEN expression can be readily assessed using immunohistochemistry. We have demonstrated a significant association between loss of PTEN expression, higher Gleason score and the Gleason Grade group. PTEN loss in Gleason score 7 can be heterogeneous and significantly vary between grade II and III. This finding may help in deciding the radical treatment in patients where clinical risk assessment sometimes is not decisive.

We also support the observation that PTEN deletion in prostate carcinoma can be used as a marker of aggressiveness of the tumor.

However, the clinical application of PTEN as a biomarker marker in prostate carcinoma needs to be validated in a large-scale study with molecular techniques.

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All the authors have contributed to concept, literature search, data acquisition, data analysis, manuscript editing and review. There was no source of funding for our research.

Conflict of interest

We declare that there are no conflicts of interest amongst the authors. The manuscript has been read and approved by all the authors, the requirements for authorship have been met and each author believes that the manuscript represents honest work, if that information is not provided in another form.

References

1. Rawla, P. (2019). Epidemiology of prostate cancer. World Journal of Oncology, 10(2), 63–89. https://doi.org/10.4021/wjon.v10i2.1191
2. Netto GJ, Amin MB. The Lower Urinary Tract and Male Genital System. In: Kumar V, Abbas AK, Aster JC, editors. Robbins & cotran pathologic basis of disease - general pathology, 10th ed. vol 2: South east asia. New Delhi, India: Elsevier; 2018. p. 975–83.
3. Yoshimoto M, Ding K, Sweet JM, Ludkovski O, Trottier G, Song KS, *et al*. PTEN losses exhibit heterogeneity in multifocal prostatic adenocarcinoma and are associated with higher Gleason Grade. Mod Pathol. 2013;26(3):435–47.
4. Jamaspishvili T, Berman DM, Ross AE, Scher HI, De Marzo AM, Squire JA, *et al*. Clinical implications of PTEN loss in prostate cancer. Nat Rev Urol. 2018;15(4):222–34.
5. Bhalla R, Kunju LP, Tomlins SA, Christopherson K, Cortez C, Carskadon S, *et al*. Novel dual-color immunohistochemical methods for detecting ERG-PTEN and ERG-SPINK1 status in prostate carcinoma. Mod Pathol. 2013;26(6):835–48
6. Gamble M. The Haematoxylins & Eosin. In: Bancroft JD, Gamble M, editors. Theory and Practice of Histologic techniques. 6th ed. Philadelphia: Churchill Livingstone; 2008:121-34.
7. Nabeel T B Kammuna, Kaswr M J Al-Taraihi. The validity of Immunohistochemical Expression of PTEN protein in detecting PTEN gene deletion in Prostate cancer. Al-Qadisiah Medical Journal. 2017;13(1):113–24.
8. Bio Medical Waste Management (Amendment) Rules 2018. New Delhi: Gazette of India, Extraordinary, Part II, Section 3, Subsection(i);2018 Mar.
9. Switlyk MD, Salberg UB, Geier OM, Vlatkovic L, Lilleby W, Lyng H, *et al*. PTEN expression in prostate cancer: Relationship with clinicopathologic features and multiparametric MRI findings. AJR Am J Roentgenol [Internet]. 2019 [cited 2022 Nov 13];212(6):1–9. Available from: https://pubmed.ncbi.nlm.nih.gov/30888866/
10. Picanço-Albuquerque CG, Vidotto T, Pereira CS, Saggioro FP, Jamaspishvili T, Koti M, *et al*. PTEN loss in Gleason Grade 7 prostate tumors exhibits intratumoral heterogeneity and is associated with unfavorable pathological features. Appl Canc Res. 2019;39(1):1.
11. Lotan TL, Carvalho FL, Peskoe SB, Hicks JL, Good J, Fedor HL, *et al*. PTEN loss is associated with upgrading of prostate cancer from biopsy to radical prostatectomy. Mod Pathol. 2015;28(1):128–37.
12. Jamaspishvili T, Patel PG, Niu Y, Vidotto T, Caven I, Livergant R, *et al*. Risk stratification of prostate cancer through quantitative assessment of PTEN loss (qPTEN). J Natl Cancer Inst [Internet]. 2020 [cited 2022 Jul 26];112(11):1098–104. Available from: http://dx.doi.org/10.1093/jnci/djaa032
13. Gordetsky J, Epstein J. Grading of prostatic adenocarcinoma: current state and prognostic implications. Diagn Pathol [Internet]. 2016 [cited 2022 Nov 13];11(1):25. Available from: https://pubmed.ncbi.nlm.nih.gov/26956509/
14. Dess RT, Morgan TM, Nguyen PL, Mehra R, Sandler HM, Feng FY, *et al*. Adjuvant versus early salvage radiation therapy following radical prostatectomy for men with localized prostate cancer. Curr Urol Rep [Internet]. 2017 [cited 2022 Nov 13];18(7):55. Available from: https://pubmed.ncbi.nlm.nih.gov/28589403/
15. Shah MD, Shore KT, Yoon J, Mendrinos MD, McKenney JK, Tian W. PTEN loss in prostatic adenocarcinoma correlates with specific adverse histologic features (intraductal carcinoma, cribriform Gleasonpattern 4 and stromogenic carcinoma). The Prostate 2019;79:1267-1273.
16. Hamid AA, Gray KP, Huang Y, Bowden M, Pomerantz M, Loda M, *et al*. Loss of PTEN expression detected by fluorescence immunohistochemistry predicts lethal prostate cancer in men treated with prostatectomy. Eur Urol Oncol. 2019;2(5):475–82.
17. Cuzick J, Yang ZH, Fisher G, Tikishvili E, Stone S, Lanchbury JS, *et al*. Prognostic value of PTEN loss in men with conservatively managed localised prostate cancer. Br J Cancer[Internet]. 2013 [cited 2022 Jul 26]; 108(12):2582–9. Available from: https://pubmed.ncbi.nlm.nih.gov/23695019/