ORIGINAL RESEARCH

Histopathological spectrum of prostatic lesions and correlation with immunohistochemical marker expression in selected cases

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ABSTRACT

Introduction: Prostate is one of the most commonly affected organ in males with increasing age. Prostatic lesions like Benign Prostatic Hyperplasia, Prostatic Adenocarcinoma account for significant morbidity and mortality. Though the diagnosis of malignant tumours of prostate can be made with morphological features, it is sometimes very difficult to reach a definitive diagnosis by routine histopathological examination alone.Therefore, the Immunohistochemistry is very useful in distinguishing the prostate cancer from benign lesions, especially in equivocal cases. Based on this aim of our study is to study the spectrum of various Prostatic lesions and also to study the expression of Immunohistochemical markers P63 and AMACR in benign and malignant cases. **Materials and methods:**The present study is a prospective study of prostate biopsies of 115 clinically suspected cases of prostatic lesions who were reported in the Department of Urology, in our institution during the period from December 2020 - June 2022. All these 115 biopsies were subjected to histopathological examination and categorized into benign and malignant lesions. Histological grading was done using Gleason's scoring system for all the prostatic carcinoma cases. Immunohistochemical examination was done using the immunostains P63 and AMACR in selected cases. **Results:** In our study, p63 has sensitivity of 100%, specificity of 100% and diagnostic accuracy of 100% where as AMACR has sensitivity of 100%, specificity of 86.67% and diagnostic accuracy of 93.33%. P value of less than 0.05% was considered significant. Association of type of lesion with P63 and AMACR was significant with p value < 0.001. **Conclusion:**The histopathological examination is still the gold standard modality for the diagnosis of prostatic lesions, Immunohistochemistry with p63 and AMACR in the assessment of prostatic lesions, is of immense additional value in further confirming the histomorphologically diagnosed prostatic benign and malignant cases.

Keywords: AMACR, P63, Prostate biopsy, suspicious prostatic foci.

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INTRODUCTION

Prostate is one of the most commonly affected organ in males with increasing age. Prostatic lesions like Benign Prostatic Hyperplasia (BPH), Prostatic Adenocarcinoma account for significant morbidity and mortality.[1,2] Prostate cancer is the second most common cause of cancer in men, next only to the lung cancer. The incidence of prostate cancer varies widely throughout the world. There has been a gradual increase in the incidence of prostate cancer since the 1960s in many countries; there were large increasein the late 1980s and early 1990s, but increases have also occurred in countries with comparatively low incidence.[3] Globally, the number of new cases in 2020 including all ages is 14 lakhs approximately (7.3% of all cancers).[4]

The environmental exposures and genetic factors, both contribute to marked differences in the incidence of prostate cancer across the world. Some genetic factors increase the risk due to intrinsic effects on prostatic epithelium while other factors act by modifying the risk associated with the environmental exposures.[1,2]

Due to the widespread use of serum Prostate Specific Antigen (PSA) along with imaging studies, the incidence of prostate cancer is markedly increased. The interpretation of the small biopsies is often very difficult due to either a small focus of malignancy or due to the presence of benign lesions mimicking malignancy.[5] Accurate diagnosis of the small biopsies is very important because if diagnosed early, patient

will be benefitted by avoiding radical procedures with significant mortality and morbidity.

Though the diagnosis of malignant tumours of prostate can be made with morphological features like pattern, nuclear atypia and absence of basal cells, it is sometimes very difficult to reach a definitive diagnosis by routine histopathological examination alone.6 Therefore, the Immunohistochemistry is very useful in distinguishing the prostate cancer from benign lesions, especially in equivocal cases.In this study an attempt has been made to study the various prostatic lesions by Histopathology and Immunohistochemistry in selected cases. Therefore our aim was to study the spectrum of various Prostatic lesions and also to study the expression of Immunohistochemical markers P63 and AMACRin benign and malignant cases.

MATERIALS AND METHODS

This study was done at department of Pathology, in our institution as a prospective study in the period of December 2020 to June 2022. Ethical clearance for the study was obtained from the Institutional Ethics Committee of our institution. A total sample of 115 cases of prostatic lesions was analyzed during the period. Tissue blocks of all prostate biopsies which were received during thestudy periodas benign and malignant prostatic lesions were included on the study. Tissue blocks of patients who were diagnosed as prostatic carcinoma but underwent preoperative Chemotherapy or Radiotherapy were excluded.

Tissue sections prepared from paraffin embedded formalin fixed tissues. Haematoxylin and eosin staining kit P63 and AMACR monoclonal antibody kit and Secondary antibody kit was used.

Formalin fixed paraffin embedded blocks and haematoxylin eosinstained sections of 115 prostatic biopsies which included TURP, Trucut biopsy, TRUS biopsy and open prostatectomy specimens were selected for the study. Based on the histopathological examination, they were categorized as benign and malignant lesions. Prostatic adenocarcinoma was assigned Gleason grade ranging from grade 1 to grade 5 according to the modified Gleason grading system. Immunohistochemistry was performed on the tissue sections along with positive and negative control.

The IHC sections were studied for expression of P63 and AMACR. IHC results were considered positive, in case of dark, diffuse or granular, cytoplasmic or luminal staining and which can be easily observed at the low power(<100X) . IHC staining was scored as follows: positive as 1, and negative as 0.A positive immunostaining for p63 is defined as positive staining of the nuclei of basal cells. Positive staining in the foci in question is taken as the evidence of benignity and negative staining of entire suspicious foci is taken as presumptive evidence of malignant process. Both the positive and negative controls were put with every batch of IHC slides for correctness of the procedure. IHC staining was scored as follows: positive as 1, and negative as 0.

The primary data was entered in MS Excel and analyzed using SPSS 20v. The results were presented in the form of tables and graphs. The descriptive statistics frequency and their percentages were calculated. The association between the categorical variables were analyzed by Chi square test with 5% level of significance.

RESULTS

The present study is a prospective study of prostate biopsies of 115 clinically suspected cases of prostatic lesions who were reported in the Department of Urology, in our institution during the period from December 2020 - June 2022. All these 115 biopsies were subjected to histopathological examination and categorized into benign and malignant lesions. Histological grading was done using Gleason's scoring system for all the prostatic carcinoma cases.Immunohistochemical examination was done using the immunostains P63 and AMACR in selected cases.

In our study the mean age of the patients was 67.92 years with a standard deviation of9.31 years. The range of age group of the patients was between 43 years to 95 years population. In subjects with benign disease, maximumproportion of patients- 47 cases (48.5%) were in the age group 65 to74years. In subjects with malignancy,maximum proportionof patients - 8 cases (44.4%) were also in the age group 65to 74 years. The difference in proportion of age group and type of lesions between the two groups was statistically not significant(p-value = 0.385)

Theclinicaldiagnosisofthepatientswas classified into tothefollowingcategories as per the distribution shown in below table. Among the study population, the commonest clinical diagnosis was BPH with a frequency of83 cases. Most of the cases in the study population had the ultra-sonogram finding of Grade II Prostatomegaly (40%). Out of the 18 cases of Grade IV Prostatomegaly, 10 cases were histopathologically diagnosed as Prostatic Adenocarcinoma and one case was HGPN.

Table 1: Descriptive analysis of Clinical diagnosis

Clinical Diagnosis	Frequency	Percent
BPH	83	72.2%
BPH with raised PSA	9	7.8%
Prostatic Abscess	1	0.9%
Suspected Carcinoma Prostate	14	12.2%
Carcinoma Prostate	5	4.3%

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Carcinoma Prostate with bone metastasis	1	0.9%
Carcinoma Bladder with prostatomegaly	2	1.7%

Serum PSA Level was less than 10 ng/ml in 43 cases, 11 to 50 ng/ml in 46 cases, 51 to 100 ng/ml in one case, 101 to 500 ng/ml in 21 cases and more than 500 ng/ml in 4 cases. All the 25 cases of Prostatitis showed mildly increased levels of serum PSA levels. Out of the 18 cases of Prostatic Adenocarcinoma in our study, 15 cases showed elevated PSA levels. Very high levels of PSA (>500 ng/ml) were seen in Prostatic Adenocarcinoma. Among the types of specimens received, the TURP biopsies were maximum with a frequency of 88 biopsies (76.6%)in our study population.

Among the study population, the Prostatic Adenocarcinomaswere18 cases (15.7%).Premalignant (HGPIN) lesions were 2 cases (1.7%). Benign and inflammatorylesions together constituted95 cases (82.6%). All the 18 cases of Prostatic Adenocarcinomas in our study population were Acinar type Adenocarcinoma.

Out of the 84 cases clinically diagnosed as benign lesions (BPH and Prostatic abscess with normal PSA levels), 11 cases (13.10%) were histopathologically diagnosed as Prostatic Adenocarcinoma, and other 73cases (86.90%) were diagnosed as benign lesions. Out of the 9 cases clinically diagnosed as BPH but with increased PSA levels, 3 cases (33.33%) were histopathologically diagnosed as Prostatic Adenocarcinoma; other 6 cases (66.67%) were diagnosed as benign lesions.

Out of the 14 cases clinically suspected to have Carcinoma Prostate, 1 case (7.14%)was histopathologically diagnosed as Nodular Hyperplasia of Prostate, 2 cases (14.28%)were diagnosed as Nodular Hyperplasia of Prostate with associated Prostatitis, 4 cases (28.57%) were interpreted as Fibromuscular stroma only, 7 cases (50%) were diagnosed as Prostatic Adenocarcinoma.

Out of the 5 patients clinically diagnosed to have Carcinoma Prostate, all 5 cases (100%) were histopathologically diagnosed as Prostatic Adenocarcinoma.Out of the 2 patients clinically diagnosed to have Carcinoma Bladder with Prostatomegaly, all 2 cases (100%) were histopathologically diagnosed as HGPIN.One patient, who was clinically diagnosed to have Carcinoma Prostate with bone metastasis, was histopathologically diagnosed as Prostatic Adenocarcinoma. Among the study population, Prostatitis was present in 27 cases (23.48%) and was absent in88 cases (76.52%) of our study population.

The Gleason's system of scoring was applied in all the 18 cases of Prostatic Adenocarcinoma. The predominant proportion of Adenocarcinomas was grouped under Gleason Grade group 4 - 6 cases (33.33%).

Table 2:Gleason Scorein Prostatic Adenocarcinoma (N=18)

Gleason Grade Group	Gleason score	No.of cases	Percentage
1	3+3	1	5.56%
2	3+4	4	22.22%
3	4+3	3	16.67%
4	4+4 3+5 5+3	6	33.33%
5	5+4 5+5	4	22.22%

CORRELATION OF HISTOPATHOLOGY WITH P63 STAINING

P63 immunostaining was done for 30 selected cases (13 benign cases, 2 HGPIN and 15 malignant cases) among the study population. P63 Positive was positive in all the 13 benign cases (100%).All the 6 cases of Nodular Hyperplasia of Prostate with the associated focus of benign mimics expressed P63 in the basal cells. Two HGPIN cases showed P63 positivity in the basal cells.In 2cases of Nodular Hyperplasia of Prostate with a focus of few irregular glands suspicious of malignancy, P63 immunostaining was done and found to be positive in the basal cells and thereby confirmed the benignity.P63 was negative in all the 15 malignant cases (100%). There was a strong correlation of the P63 staining with the histopathological diagnosis. A total of 2cases were included in the analysis which was found to be suspicious of malignancy on histomorphology due to the presence of irregular glands.

Table 3: Descriptive analysis of P63(N=30)

P63 immunostaining	Histopathological Patterns		Percentage
	Benign& HGPIN	Malignant	
Positive	15	0	100%
Negative	0	15	100%

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Sensitivity of P63 in diagnosing Benign lesions was 100%, Specificity of P63 in diagnosing Benign lesions was 100% .Positive predictive value of P63 in diagnosing Benign lesions was 100% , Negative predictive value of P63 in diagnosing Benign lesions was 100% and the total diagnostic accuracy of P63 in diagnosing Benign lesions was 100% .

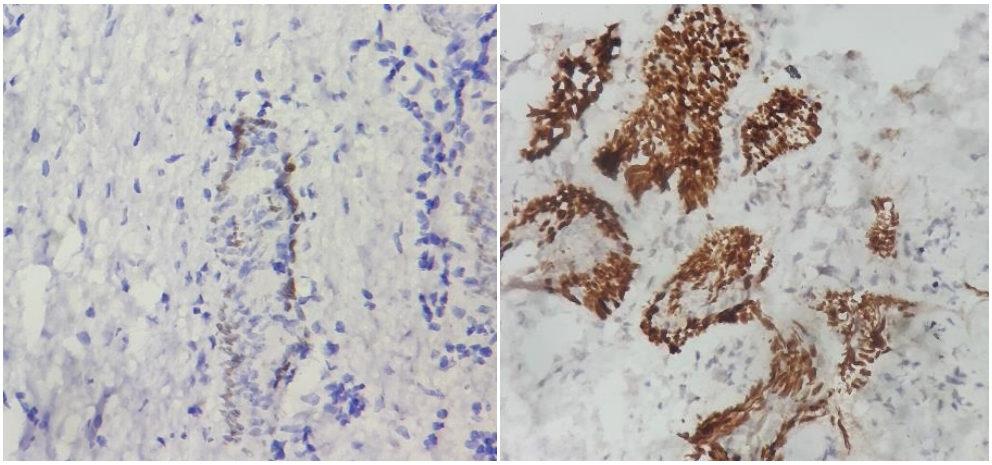

Figure 1& 2 - P63 Positive in irregular glands in BPH and in Basal cell hyperplasi .

CORRELATION OF HISTOPATHOLOGY WITH AMACR STAINING

The AMACR staining was expressed as cytoplasmic granular positivity in all the 15 cases of malignancies and 1 case of HGPIN. It was found to be false positive in one case of histopathologically and immunohistochemically confirmed benign prostatic hyperplasia. It wasfound to be negativein 12cases of benign prostatic hyperplasia and 1 case of HGPIN. There was a strong correlation of the AMACR staining with the histopathological diagnosis of Prostatic Adenocarcinoma. Amongthe 30 selected cases of study population subjected to immunohistochemical analysis, AMACR Positivity was seen in 17 cases (56.67%) and Negativity was seen in 13 cases (43.33%), including the focus of benign mimics of 6 cases.

Table 4: Descriptive analysis of AMACR staining

AMACR	Histopathological Pattern		
	Benign	HGPIN	Malignant
Positive	1 (7.69%)	1 (50%)	15 (100%)
Negative	12 (92.31%)	1 (50%)	0

Sensitivity of AMACR in diagnosing malignant cases was 100%, Specificity of AMACR in diagnosing malignant cases was86.67%, and Positive predictive value of AMACR in diagnosing malignant cases was 88.24%. Negative predictive value of AMACR in diagnosing malignant cases was 100% and the total diagnostic accuracy of AMACR in diagnosing malignant cases was 93.33%.

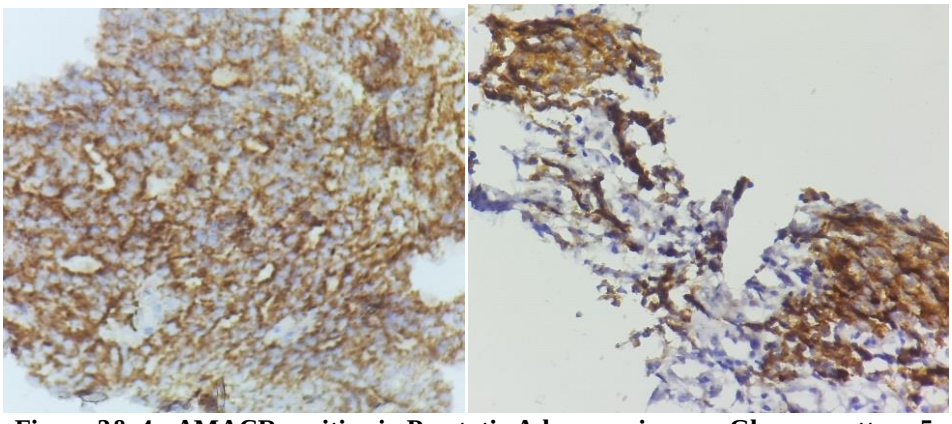

Figure 3& 4 : AMACR positive in Prostatic Adenocarcinoma , Gleason pattern 5

In 15 cases of malignancies, the AMACR staining was seen in all 15 cases of Prostatic Adenocarcinomas irrespective of Gleason score. There was no correlation between the AMACR staining and the histopathological Gleason's grade.

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DISCUSSION

Benign Prostatic Hyperplasia (BPH) and Prostatic carcinoma are the two principle conditions involving the prostate, accounting for more than 90% of all prostatic diseases. Prostatic cancer causes a significant health problem in the world, being the second most common cancer in men. It is relatively rare for the prostate cancer to be diagnosed in men less than 50 years of age, but after 50 years of age, the incidence and mortality rates increase exponentially[6] Benign Prostatic Hyperplasia usually begins before 30 years of age and approximately 8% of men are having histological evidence of Benign Prostatic Hyperplasia by 40 years of age, 50% by 60 years of age and 90% by 90 years of age.

In our study, most common age group of patients with BPH and Prostatic adenocarcinoma was 65 - 74 years. This is similar to the study by Monika Garg et al. in which the average group was 68.6 years,[7] whereas in a study by ArchanaC.Buch et al. most of the patients were in the age group of 71 to 79 years.[8] The difference in the proportion of age group and the type of lesion between the two groups were statistically not significant (p-value = 0.385) in the current study.

In our study, the commonest clinical diagnosis was BPH which is similar to the literature.[9] Nowadays, PSA is the commonly used serum marker. There is enough evidence that widespread PSA screening and hence early detection of cancer resulted in decreased mortality rate associated with decline in the metastatic rate. However, PSA test and associated analysis have been criticized from the beginning and controversies are still unresolved. In our study, out of the 18 cases of Prostatic Adenocarcinoma, 15 cases showed elevated serum PSA levels. All the 25 cases of Prostatitis showed mildly increased levels of serum PSA levels.

The commonest histopathological spectrum in our study was Nodular Hyperplasia of Prostate - 53.9% (Benign Prostatic Hyperplasia), followed by Nodular Hyperplasia of Prostate with Prostatitis - 21.7%. In a study by Archana C. Buch et al. the commonest histopathological spectrum was Nodular Hyperplasia of Prostate with Prostatitis.[8]

Benign microscopic mimics of Prostatic carcinoma impose diagnostic dilemma. In the current study, in 6 cases of BPH, the following benign mimics were seen in adjacent foci. Basal cell hyperplasia - 2 cases, Adenosis, Clear cell hyperplasia, Urothelial metaplasia and clusters of Foamy histiocytes each constituting 1 case each.

Mahapatra et al. studied various mimickers of Prostatic carcinoma and basal cell hyperplasia was the most common mimic in his study.[10]In the study by AkshayBondge et al. Basal cell hyperplasia was the only mimicker found among the 61 benign lesions diagnosed.[11] Both the studies coincide with our study.

The mean incidence rate of the isolated high-grade PIN is 9% (range 4% to 25%) of prostate biopsies, 16% in the needle core biopsies and 1-5% in TURP.[12,13]In our study, the incidence rate of isolated high-grade PIN is 1.74% (2 cases).All the Adenocarcinoma were graded according to Gleason's grading system. In our study maximum number of cases were of Gleason's grade group 4 (33.33%).

A Gleason score of 7 is associated with a critical decision-making step and these patients usually need some form of definitive therapy. Patients with Gleason score of 8–10 are often suitable candidates for adjuvant therapy or radiation therapy. We have used the two monoclonal antibodies in combination to solve the cases with atypical/suspicious foci. In our study, we had 2 cases (1.74%) with suspicious foci. In various studies in the literature, the incidence of atypical biopsies ranges from 0.4 - 23% with a mean of 5.5%.[14]

In the current study, out of 115 cases, 30 cases were subjected to Immunohistochemical analysis with p63 and AMACR. In 11 histologically confirmed benign cases, P63 was expressed in basal cells of all the BPH lesions. In 2 cases of BPH with a focus of irregular glands suspicious of malignancy, P63 expression was found to be positive in the nuclei of basal cells and thus confirmed the benignity. Two cases of HGPIN also showed P63 positivity and thereby ruled out invasiveness. The sensitivity and specificity of P63 marker in our study is 100%. In a study by AnuradhaG.Patil et al, diagnostic accuracy of p63 was 96.15% and the ability of P63 to distinguish between Prostatic carcinoma and non carcinomatous lesions were statistically significant.[15]

In our study, AMACR was significantly expressed in all the 15 cases of Prostatic Adenocarcinoma (p value - 0.000). AMACR expression was present in one case of HGPIN and negative in the other case of HGPIN. AMACR expression in HGPIN lesions was not statistically significant. In a histopathologically confirmed case of BPH, which was also positive for P63 expression in basal cells, there was AMACR expression and it was found to be false positive. All the other 12 benign lesions including those cases of benign mimics were found to be negative for AMACR. Therefore, Sensitivity and Positive predictive value of AMACR expression in malignant lesions were 100% and 88.24% respectively. In benign lesions, Specificity and Negative predictive value of AMACR expression were 86.67% and 100% respectively.

In a study by A.Mohamedet al.[16] AMACR had 95% sensitivity and 98% specificity. Statistically, AMACR expression was significant in Prostatic Adenocarcinomas with p value (0.000), which coincides with our study.

In a study by Sangeetha Kandhasamyet al.[17] 91% of malignant cases showed AMACR positivity, whereas all benign cases showed AMACR negativity. Sensitivity and Specificity of AMACR in Prostatic carcinoma were 90% and 100% respectively. Positive predictive value of AMACR was 100% and Negative predictive value of AMACR was 65%.

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Hence, AMACR helps to confirm the diagnosis of malignancy by positive signal. P63 positivity and AMACR negativity in the 6 cases of BPH with focus of benign mimics, aided in confirming the diagnosis of benignity.

CONCLUSION

There are challenges in the diagnosis of Prostatic lesions, when evaluation is done on limited tissue. We have to carefully consider and rule out various benign lesions that mimic carcinoma. Correct diagnosis and grading of Prostatic carcinoma is crucial for patient's prognostic and therapeutic options. The histopathological examination is still the gold standard modality for the diagnosis of prostatic lesions, Immunohistochemistry with p63 and AMACR in the assessment of prostatic lesions, is of immense additional value in further confirming the histomorphologically diagnosed prostatic benign and malignant cases and also for evaluating the morphologically suspicious cases and it should be used on a case to case basis especially in the needle biopsies and small foci lesions.

REFERENCES

1. Prostate Cancer Causes, Risk Factors, and Prevention. American Cancer Society; cancer.org | 1.800.227.2345
2. Risk Factors for Prostate Cancer Peter H Gann, MD, ScD Rev Urol. 2002; 4(Suppl 5): S3–S10.
3. Quinn M, Babb P. Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part I: international comparisons. BJU Int. 2002 Jul;90(2):162-73.
4. Prostate cancer Incidence: Globocan 2020, International agency for Research on Cancer, WHO.
5. Prensner JR, Rubin MA, Wei JT, Chinnaiyan AM. Beyond PSA: the next generation of prostate cancer biomarkers. SciTransl Med. 2012 Mar 28; 4(127):127rv3.
6. American cancer society. Available from htttp//www.cancer.org.
7. Garg M, Kaur G, Malhotra V, Garg R. Histopathological spectrum of 364 prostatic specimens including immunohistochemistry with special reference to grey zone lesions. Prostate Int. 2013; 1(4):146-51. doi: 10.12954/PI.13026. Epub 2013 Dec 30.
8. Archana C. Buch et al (2021). Histopathological Spectrum of Lesions of Prostate. Saudi J PatholMicrobiol, 6(6): 229-233.
9. Aslam, H. M., Shahid, N., Shaikh, N. A., Shaikh, H. A., Saleem, S., & Mughal, A. (2013). Spectrum of prostatic lesions. International Archives of Medicine, 6(1), 1–5.
10. Mahapatra QS, Mohanty P, Nanda A, Mohanty L. Histomorphological study of prostatic adenocarcinoma and its mimics. Indian J PatholMicrobiol 2019;62:251-60
11. Dr. Archana C Buch, et. al. "The Utility of PSA and PSA Density in Assessing the Risk of Prostate Cancer." IOSR Journal of Pharmacy (IOSRPHR), 11(03), 2021, pp. 46-50.
12. David G. Bostwick, 8 - Nonneoplastic Diseases of the Prostate, Urologic Surgical Pathology, Fourth Edition, Elsevier, 2020, Pages 358-414.e13
13. WHO Classification of Tumors of the Urinary System and Male Genital Organs 4th edition (Holger Moch, Peter A. Humphrey et al)
14. Kumaresan K, Kakkar N, Verma A, Mandal AK, Singh SK, Joshi K. Diagnostic utility of α-methylacyl CoA racemase (P504S) & HMWCK in morphologically difficult prostate cancer. DiagnPathol. 2010 Dec 22; 5:83.
15. AnuradhaG.Patil, DebarghyaSutradhar, AnuTresa Antony, & Anita A.M. (2022). Immuno- Histochemical Study of p63 Expression in Prostatic Lesions. International Journal of Health and Clinical Research, 5(3), 522-527.
16. Ahmed Ibn Edriss Mohamed, El Jaily Mohamed, Abbashar Mohamed, Mohamed El Imam, "*Optimizing Detection of Prostate Cancer by AMACR and P63 on Prostatic Needle Biopsy- Sudanese Experience*", International Journal of Science and Research (IJSR), Volume 9 Issue 3, March 2020, pp. 1580-1583.
17. Kandasamy, Sangeetha&Pothen, Lillykutty& V, Letha &Sankar, Sundar& V, Priya& Varghese, Sheela &Leelamma, Jayalakshmy. (2017). DIAGNOSTIC UTILITY OF AMACR IN PROSTATIC CARCINOMA. Journal of Evolution of Medical and Dental Sciences. 6. 2869-2873.

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