VOLUME - 11, ISSUE - 11, NOVEMBER - 2022 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Pathology

COMPARISON OF BASAL-CELL SPECIFIC MARKERS – P63 AND 34BETAE12 IN PROSTATIC GLANDULAR PROLIFERATIONS

Dr. Ningthibi Jessica Akoijam	Senior Resident, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur
Dr. Laishram Deepak Kumar	Assistant Professor, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Sushma Khuraijam	Professor and Head of Department, Department of Pathology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Akoijam Kaku	Professor and Head of Department, Department of Urology, Regional Institute of Medical Sciences, Imphal, Manipur.
Dr. Alpana Laisom*	Professor and Head of Department, Department of Urology, Regional Institute of Medical Sciences, Imphal, Manipur. *Corresponding Author

ABSTRACT

Background: The prostate is a retroperitoneal organ encircling the neck of the bladder and urethra. Though the diagnosis of the prostatic lesions are analyzed through histopathological examination (HPE), sometimes, diagnosis can be challenging, when pathologist are faced with certain problems such as small foci of Ca or benign mimickers. In such situation, immunohistochemical (IHC) detection of basal cells are widely used. **Objectives:** To assess the expression of basal cell markers (p63 and 34betaE12) in various prostatic glandular proliferations and to differentiate suspicious glandular lesions as benign or malignant. **Methods:** A two year cross-sectional study (Sept'2016 –Aug'2018) , total of 52 cases of both TURP and prostate biopsy specimens sent to the department of Pathology, RIMS for HPE were studied using IHC markers p63 and 34betaE12, following H&E stain and the expressions of the markers were studied. Results: Out of 52 cases, 41(78.8%) cases were diagnosed as Benign proliferative hyperplasia (BPH), 8(15.4%) cases as prostatic carcinoma, 2(3.8%) cases as high grade prostatic intraepithelial neoplasia (HGPIN) and one (1.9%) case of adenoleiomyofibromatous hyperplasia (AMFH) on H&E section with age range of 51 to 90 years (mean age: 72 years). Following IHC staining, 43 (97.7%) benign cases were positive for both p63 and 34betaE12, one (2.3%) case of benign lesion was negative for both the IHCs. 8(100%) cases of malignant lesions were negative for both the IHCs. A p-value of 1.000 was observed indicating that there is no significant difference in the sensitivity of p63 and 34betaE12. **Conclusion:** In this cross-sectional study of 52 cases of prostatic lesions, HPE and the role of basal cell specific IHC markers p63 and 34beta12 were studied. No significant difference was observed in the sensitivity between the two markers. Further comparative study with larger sample size is needed to comprehend the differences in the utility of p63 and 34etaE12 in diagnosing suspicious prostatic lesions.

KEYWORDS : Immunohistochemistry (IHC), p63 , 34betaE12, high grade prostatic intraepithelial neoplasia (PIN), prostatic carcinoma, adenoleiomyofibromatous (AMFH)

INTRODUCTION

The prostate gland is a retroperitoneal organ and develops from epithelial invaginations from the posterior urogenital sinus under the influence of the underlying mesenchyme, during the third month of gestation. It has three major zones-peripheral zone, central zone and transition zone. The histologic architecture of the prostate is that of a branched duct gland. Two cell layers, a luminal secretory columnar cell layer and an underlying basal cell layer, line each gland or duct.[1]

Benign prostatic hyperplasia (BPH) is the most common benign prostatic disease in men older than age 50 years. It results from nodular hyperplasia of prostatic stromal and epithelial cells and often leads to urinary obstruction and mostly arises from the transitional zone.[2] Histologically, the glandular component is made up of nodules of small and large acini lined by basal and secretory cells. Some glands show papillary infoldings and projections. The stromal component often shows both fibrous and smooth muscle elements.[3]

Prostatic intraepithelial neoplasia (PIN), as a precursor to some prostatic carcinomas, was first described in the 1960s by McNeal under the name of 'intraductal dysplasia', and was more precisely characterized in 1986 by McNeal and Bostwick[4] Microscopically, PIN is easily distinguished from normal or hyperplastic glandular epithelium on low-power magnification because the affected glands or ducts most often depict striking hyperchromasia and nuclear stratification. On low-power examination the triad of too dark (hyperchromatic), too thick (nuclear stratification), and too complex (luminal complexity) should raise suspicion for PIN. On high-power examination, the triad of nucleomegaly, prominent nucleoli, and hyperchromasia are seen.[5]

Prostate cancer is the world's leading cause of cancer and the second cause of cancer-related death in men after lung cancer. Cancer of the prostate is typically a disease of men over age 50.[2] Conventional acinar adenocarcinoma represents over 90% of prostate carcinomas.[6].

Histopathologicaly, the principal criteria for diagnosis of well differentiated adenocarcinoma include a small-gland proliferation recognized as being discrete or focally infiltrative on low power examination, the presence of a single cell lining with complete absence of the basal cell layer, nucleomegaly, and presence of large nucleoli. The size of the nucleoli is critical; in carcinoma, nucleoli are often at least 1 micron in diameter, prominent nucleoli can be recognized by their distinct cherry red color on medium-power (10× or 20×). A single cell lining (i.e., lack of basal cell layer) is also a requisite for the diagnosis of well differentiated adenocarcinoma of the prostate.[5]

Histopathological diagnosis of prostatic lesions sometimes,

can be challenging, when pathologist are faced with certain problems such as small foci of Ca or benign mimickers. In such situation, immunohistochemical (IHC) detection of basal cells are widely used. The most commonly used basal cell specific markers are high molecular weight cytokeratin [HMWCK] and newly described basal cell marker p63, HMWCK shows cytoplasmic positivity whereas p63 shows nuclear positivity.

High molecular-weight cytokeratin (HMCK) 34betaE12 is a cytoplasmic marker that highlights intermediate cytokeratin (CK) filaments in glandular basal cells and is specific for basal cells in the prostate.

The monoclonal antibody clone 34betaE12 (also known as CK903), which targets CK1, CK5, CK10, and CK14, is the time-honoured basal cell marker used since 1985. Most laboratories do not use this marker in isolation but more commonly use it in combination with prostate cancer–specific marker like methylacyl coenzyme A (coA) racemase or with other basal cell markers.[7]

p63 antibody targets the p63 nuclear protein, which is homologous to the TP53 tumor suppressor gene and has been proven to selectively stain the basal cell nuclei. p63 is comparable to HMCK in sensitivity and specificity in needle biopsies, although some studies have suggested that p63 has better sensitivity than HMCK 34betaE12 in specimens from transurethral resections of the prostate. This differential staining may be related to alterations in antigenicity of basal cells in glands of benign prostatic hyperplasia. p63 immunostaining provides greater specificity because of its nuclear localization; the cytoplasmic staining to HMCK markers may have greater potential for nonspecific reaction.[7]

This study was carried out to study the expression of basal cell markers (p63 and 34betaE12) in various prostatic glandular proliferations and to differentiate suspicious glandular lesions as benign or malignant. And to explore their utility in corroborating the findings of HPE.[8]

METHODS

A cross sectional study spanning 2 years (October 2016 to September 2018) was conducted in the Department of Pathology, Regional Institute of Medical Sciences (RIMS), Imphal, Manipur, in collaboration with Department of Urology, RIMS, Imphal. All prostate samples obtained from prostate biopsy and TURP, sent to the Department of Pathology, RIMS from both the outpatient and inpatient for HPE in the Department of Pathology, during the study period were included in the study. Samples from post radiation therapy, post chemotherapy patients and inadequate prostatic biopsies were excluded from the study. All together 52 prostatic biopsies as well as TURP samples were received. The tissues were fixed in formalin and taken up for routine histopathological studies and IHC studies for p63 and HMWCK 34betaE12. Monoclonal mouse Anti-Human p63 protein, Dako and Monoclonal mouse Anti-Human HMWCK 34betaE12, Dako were used in all IHC analysis. Sections of 3-5 □m were cut on to poly L-lysine coated slides and were deparaffinized and rehydrated. Antigen retrieval was achieved by microwave method using Tris buffer. Slides were allowed to cool for 20 minutes and blocking reagent was applied and kept for 10 minutes.

Tissues were covered with primary antibody and were incubated for 1 hour at room temperature in humidity chamber. Polymer HRP labelled secondary antibody detection kit was added on the sections and incubated in humidity chamber for 30 minutes. DAB chromogen was added on the section for 10 minutes and then washed with D/W. All slides were counterstained with Haematoxylin, dehydrated and mounted. Between each step, the slides were washed with phosphate buffer solution (PBS). The staining proportion of basal cells was subdivided into the number of cells stained with the marker i.e., <5%, 5%-75% and >75% of cells for both p63 and 34betaE12.

Data collected was entered and analyzed using IBM SPSS Statistics 21 for Windows. Descriptive statistics like mean, standard deviation, percentage and proportion were used in variables like age, histopathological and IHC findings. Sensitivity, specificity of p63 and 34betaE12 were calculated Statistical analysis of p - value < 0.05 was considered as statistically significant at 95% confidence interval.

Ethical approval was obtained from the Research Ethics Board, RIMS, Imphal with reference number A/206/REB-Comm(SP)/RIMS/2015/207/75/2016, dated 14th March 2018. Informed consent was taken from the participants before recruitment.

RESULTS

All together 52 cases of prostatic biopsies as well as TURP were analyzed. The age ranged from 51-90 years old (mean 72.1 years) and maximum patients (24 cases, 46.2%) presented in the age group between 71-80 years (Figure 1). Out of the 52 cases, 44 (84.6%) cases were non-malignant consisting of 41 (78.8%) cases of BPH, 2 (3.8%) cases of HGPIN and 1 (1.9%) case of AMFH. Remaining 8 (15.4%) cases of prostatic adenocarcinoma constituted the malignant cases (Figure 2). The maximum number of BPH were found in the age between 61-80 years (mean: 71.3 years). Prostatic adenocarcinoma was most common in the age group 71-80 years (mean: 75.5 years).

IHCs p63 and 34betaE12 staining were done following H&E stain. Both were positive in 43 (82.7%) out of the 44 non – malignant cases which included 41 cases of BPH, 1 case of HGPIN and 1 case of AMFH. It was negative in 9 (17.3%) which includes 8 cases of prostate carcinoma and 1 case of HGPIN. The staining proportion of basal cells was subdivided into the number of cells stained with the marker i.e., <5%, 5%-75% and >75% of cells for both p63 and 34betaE12. The frequencies of each percentage is listed (Table 1 & 2). Out of the 41 cases of BPH, 29 cases were immunoreactive in 5%-75% of the basal cells, remaining 12 were immunoreactive in more than 75% of basal cells. For 34betaE12, 31 cases of BPH were immunoreactive for 5%-75% of basal cells, 10 cases for >75%, 1 case of AFMH showed >75% immunoreactivity for both p63 and 34betaE12 . 1 case of HGPIN were immunoreactive for both p63 and 34betaE12 in 5%-75%% of basal cells. Remaining 1 case was negative for p63 and 34betaE12.

All the 8 cases of prostatic adenocarcinoma were negative for both markers The sensitivity of p63 and 34betaE12 was calculated using 2x2 table (Sensitivity = 97.7%, Specificity = 100%)(Table 3,4). The sensitivity of the p63 and 34betaE12 were compared using McNemar test and was found to be 97.7%. (p value of 1.000) indicating there was no significant difference in the sensitivities between the two markers.

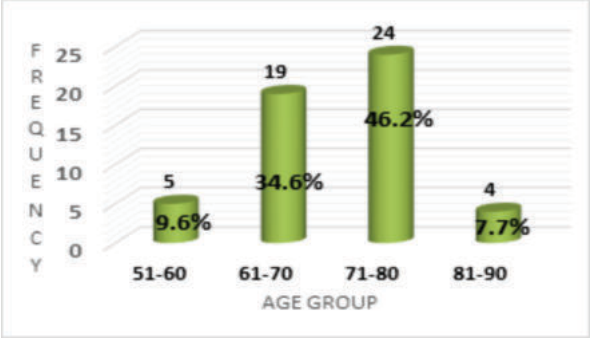

Figure 1 : Distribution of cases according to age group

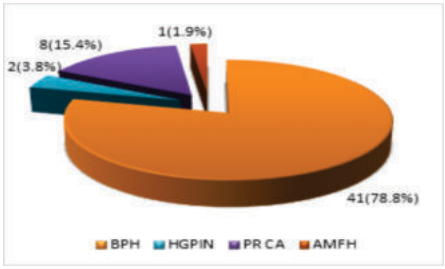

Figure. 2: Pie chart showing the distribution of cases

Table 1: Percent of cells immunoreactive for p63 in different cases

Count	p63				Total
	Diagnosis				
	BPH	PIN	Pro Ca	AMFH	
0%	0	1	8	0	9
<5%	0	1	0	0	1
5%-75%	29	0	0	1	30
>75%	12	0	0	0	12
Total	41	2	8	1	52

Table 2: Percent of cells immunoreactive for 34betaE12 in different cases

Count	34betaE12				Total
	Diagnosis				
	BPH	PIN	Pro Ca	AMFH	
0%	0	1	8	0	9
<5%	0	1	0	0	1
5%-75%	31	0	0	1	32
>75%	10	0	0	0	10
Total	41	2	8	1	52

Table : 2 x 2 table for p63

p63		Diagnosis		TOTAL
		Non malignant	Malignant	
Positive	Count	43	0	43
	%	97.70	0.00	82.70
Negative	Count	1	8	9
	%	2.30	100	17.30
TOTAL	Count	44	8	52
	%	100	100	100

Table : 2x2 table for 34betaE12

34betaE12		Diagnosis		TOTAL
		Non malignant	Malignant	
Positive	Count	43	0	43
	%	97.70	0.00	82.70
Negative	Count	1	8	9
	%	2.30	100	17.30
TOTAL	Count	44	8	52
	%	100	100	100

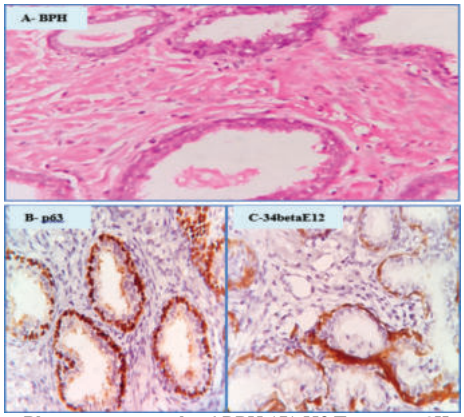

Fig.2: - Photomicrograph of BPH (A) H&E stain, 40X. (B) p63 positive in basal cells (40X, p63 IHC stain, Dako), (C) 34betaE12 positive in basal cells (40X, p63 IHC stain, Dako)

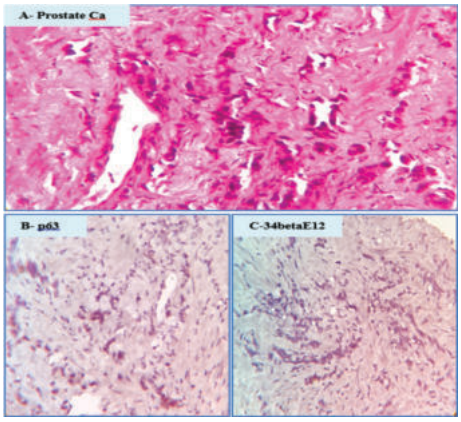

Fig.2: - Photomicrograph of Prostate carcinoma (A) H&E stain, 40X. (B) p63 negative (40X, p63 IHC stain, Dako), (C) 34betaE12 negative (40X, p63 IHC stain, Dako)

DISCUSSION

In this study, most of the prostatic lesions were seen in the age group of 71-80 years (mean : 76.8 years). Bhat et al[9] found majority of the lesions in the age group of 70-79 years (mean: 75.6 years) which is comparable to our study. In contrast, Garg et al[10], Hirachand et al[11], Aslam et al[12] found majority of the lesions between 61-70 years (mean: 68.6 years), 61-70 years (mean: 68 years) and 60-70 years (mean: 65.7 years) respectively.

The incidence of benign lesions was 80.8%, and BPH constituted 78.8% and was most frequent in the 6th - 7th decade (mean: 72.2 year). This correlates with the study done by Behera et al[13], Garg et al[10] (78.3%), Hirachand et al[11] (74.2%), Bhat et al[9] (76.6%) and Aslam et al[12] (87.5%), where the most common lesions was also BPH.

In the present study, the incidence of prostate cancer was 15. 4% most frequent in 71-80 years old (mean age: 75.5) which is comparable to the studies done by Garg et al[10], (20.1%, 71-70 years, mean age: 77.4), Hirachand et al[11] (10.1%), Bhat et al[9] (7.6%, mean age: 75.6) and Aslam et al[12] (12.5%). In contrast, Leite et al[14] found 60-70 years to be the most common age group of presentation with a mean age of 61.7 years, much younger than our observed age group.

The incidence of HGPIN in our study was 3.8% seen in the age group 71-80 years with a mean age of 75.5 years. Hirachand et al[11], Aslam et al[12] found the incidence 6.2% and 13.2% and the mean age of presentation were 66.5 and 70.2 years. In our study, majority of the cases of BPH, Prostate ca and HGPIN were presented at an older age compared to various studies. The possible explanations may be due to small sample size or study of only symptomatic cases or ignorance of the patients.

43 cases of non-malignant (41 cases of BPH, one HGPIN and one AFMH) out of a total of 44 showed positivity with both the basal markers p63 and 34betaE12. All 41 cases of BPH were positive for both the markers. This correlates with Shah et al[15], Signorette et al[16]. The results of our study demonstrates that p63and 34betaE12 are specific for basal cells in the prostate gland. One case each of HGPIN and AFMH were also immunoreactivity for the markers. This correlates with the study done by Baig MK et al[17] where they showed that p63 and 34beta12 were positive in HGPIN which is considered a mimicker of prostatic carcinoma. But one case of HGPIN reported in H&E was stained negative for both the stains. It was then reported as prostatic carcinoma due to absence of both the stains. This may be due to paucity of sample sent by

biopsy where the features of carcinoma can be missed and cases like intraductal adenocarcinoma can mimic HGPIN28. It was confirmed by negativity of both the basal cell markers. Both the markers showed immunoreactivity maximum in the range between 5%-75% of basal cells. This is contrast to the study by Kalantri et al[18] where the cases showed mostly 75% immunoreactivity. This can be attributed to may be due different manufactures of the antibody and methods of antigen retrieval during IHC staining. We use antibodies from Bio-orange Company.

A sensitivity of 97.7% was observed for both the markers which is comparable to the study by Leong Ng et al[19] where the sensitivity of both the markers was 96.5%. In our study no significant difference in the sensitivity was observed between the two markers (p value of 1.00) which is comparable to the study by Shiran et al[20] and Behera G et al[13] which studied the role of 34betaE12 and p63 in diagnosis of prostate carcinoma and observed that there was no significant difference in the sensitivity of staining pattern of both the markers. Weinstein MH et al[21] also found that the staining pattern of both the markers were comparable which correlates with our study. In contrast, Shah RB et al[22], Engelman et al[23], Samundeswari et al[24], Ali TZ et al[25] and Baig MK et al[17] observed that p63 is more sensitive than 34betaE12 in detecting prostate carcinoma and distinguishing benign lesions from malignant. This contradictory studies may be attributed due to a smaller number of atypical cases in our study which requires markers for confirmation, also due to absence if crush artifacts in our slides which was the main reason for false negativity of 34betaE12, thereby making p63 more sensitive as it is immunoreactive even when crush artifacts are present. Therefore from this study, no significant difference in the sensitivity was observed between the two basal cell markers p63 and 34betaE12. Both are equally sensitive in detecting the basal cell layer which is required in distinguishing benign lesions from malignant ones.

CONCLUSION

Prostate cancer is the world's leading cause of cancer and the second cause of cancer-related death in men after lung cancer and typically presents in men over age 50. In our study, 43 out of the 44 cases of non-malignant cases (41 cases of BPH, 1 case of HGPIN and 1 case of AFMH) showed immunoreactivity with both the markers p63 and 34beta E12. All the 8 cases of prostatic carcinomas were negative for both the markers. The sensitivity and specificity of both the markers were 97.3% and 100% respectively with a p – value of 1.000 implying that there is no significant difference in the sensitivities of both the markers . Therefore, these markers are immunoreactive in benign lesions while negative in malignant cases. From the result of this study and similar other studies we recommend the use of basal cell markers p63 and 34betaE12 as an adjunct to histological examination as they help in distinguishing benign or malignant lesions especially in morphologically difficult cases such as mimics of prostate carcinoma. Thereby, preventing over diagnosis, provide a more accurate diagnosis and help in better patient management.

Conflict of Interest

There are no conflicts of interest.

REFERENCES:

1. Hammerich KH, Ayala GE, Wheeler TM. Anatomy of the prostate gland and surgical pathology of prostate cancer. In: Hricak H, Scardino PT, editors. Prostate cancer. Cambridge:Cambridge University Press;2009.p.1-14.
2. Epstein JI and Lotan TL. The Lower Urinary Tract and Male Genital. In: Kumar V, Abbas AK, Aster JC, editors. Robbins & Cotran Pathologic Basis of Disease. 9th ed. South Asia:Elsevier;2014.p.982.
3. Epstein JJ and Netto GJ. The Prostate and Seminal Vesicles. In: Mills SE, editors. Sternberg's Diagnostic Surgical Pathology. 5th ed. Philadelphia: Wolters Kluwers;2010.p.1870.Philadelphia:Wolters Kluwers; 2010.p.1870.
4. Montironi R, Mazzucchelli R, Beltran AL, Scarpelli M, Cheng L. Prostatic intraepithelial neoplasia: its morphological and molecular diagnosis and clinical significance. BJU Int 2011;108:1394-1401.
5. Ro JY, Kim KR, Shen SS, Amin MB, Ayala AG. Tumor and Tumor like Conditions of the Male Genital tract. In: Fletcher CDM, editor. Fletcher's Diagnostic Histopathology of Tumors. Philadelphia:Elsevier;2013.p.873-74.
6. Kalantri MR, Anvari K, Jabbari H, Tabrizi FV. p63 is more sensitive and specific than 34betaE12 to differentiate adenocarcinoma of prostate from cancer mimickers. Ira J Basic Med Sci2014;17(7):497-501.
7. Paner GP, Luthringer DJ, Amin MB. Best Practice in Diagnostic Immunohistochemistry. Arch Pathol Lab Med 2008; 132:1388-96.
8. Zhou M, Shah R, Shen R, Rubin MA. Basal cell Cocktail (34betaE12 +p63) Improves the detection of Prostate Basal Cells. Am J Surg Pathol 2003;27(3):365-71.
9. Bhat S, Chaudhri S, Bhat P, Hatwal D. Histopathological Study of Prostatic Diseases in Garhwal Region. Int J Sc Study 2015;3(8):136-40.
10. Garg M, Kaur, Malhotra V, Garg R. Histopathological spectrum of 364 prostatic specimens including immunohistochemistry with special reference to grey zone lesions. Prostate Intl 2013;1(4):146-51.
11. Hirachand S, Dangol UMS, Pradhanang S, Acharya S. Study of prostatic pathology and its correlation with prostate specific antigen. J Pathol Nep 2017;7:1074-77.
12. Aslam HM, Shahid N, Shaikh NA, Shaikh HA, Saleem S, Mughal S. Spectrum of prostatic lesions. Intl Arch 2015;6(36):1-5.
13. Behera G, Mohanty L, Pujari S, Behera SK, Behera JK, Mohanty S. Diagnostic Role of Immunohistochemistry in Mimics of Prostatic Adenocarcinoma. Int J Med Res Prof 2018 Jan;4(1);61-70.
14. Leite KRM, Srougi M, Sanudo A, Dall'oglio MF, Nesrallah A, Antunes AA et al. The Use of Immunohistochemistry for Diagnosis of Prostate Cancer. International Braz J Urol 2010;36(5):583-90.
15. Shah RB,Kunju LP, Shen R, LeBlanc M, Zhou M, Rubin MA. Usefulness of Basal Cell Cocktail (34betaE12 +p63) in the Diagnosis of Atypical Prostate Glandular Proliferation. Am J Clin Pathol 2004;122:517-23.
16. Signoretti S, Waltregny D, Dilks J, Isaac B, Lin D, Garraway L et al. p63 Is a Prostate Basal Cell Marker and is Required for Prostate Development. Am J Pathol 2000;157(6):1769-75.
25. Baig MK, Hassan U, Mansoor S. Role of p63 in Differentiating Morphologically Ambiguous Lesions of Prostate. J Coll Physicians Surg Pak 2012; 22 (12): 773-77.
18. Kalantri MR, Anvari K, Jabbari H, Tabrizi FV. p63 is more sensitive and specific than 34betaE12 to differentiate adenocarcinoma of prostate from cancer mimickers. Ira J Basic Med Sci2014;17(7):497-501
19. Leong Ng VW, Koh M, Tan SY,Tan PH. Is Triple Immunostaining With 34E12, p63, and Racemase in Prostate Cancer Advantageous? Am J Clin Pathol 2007;127:248-53.
20. Shiran MS, Tan GC, Sabariah AR, Rampal L, Phang KS. p63 as a Complimentary Basal Cell Specific Marker to High Molecular weight–Cytokeratin in Distinguishing Prostate Carcinoma from Benign Prostate Lesions. Med J Pathol 2007;62:36-9.
21. Weinstein MH, Signoretti S, Loda M. Diagnostic Utility of Immuno histochemical Staining for p63,a sensitive Marker of Prostatic Basal Cells. Mod Pathol 2002;15(12):1302-08.
22. Shah RB, Zhou M, LeBlanc M, Michele SB, Synder M, Rubin M. Comparison of the Basal Cell-Specific Markers,34betaE12 and p63 in the Diagnosis of Prostate cancer. Am J Surg Pathol 2002 ;26(9):1161-68.
23. Engelman MFB, Mundim FGF, Grand MR, Carvalho LRB, Ridolfi FM. Immunohistochemistry contribution to the diagnosis of prostate cancer. J Bras Pathol Med Lab 2012;48(4):273-80.
24. Samundeswari U, Gayathri. G. The Role And Comparative Study of P63 And Hmwck In Prostatic Lesions. J Den & Med Sc 2017;16(5):87-90.
25. Ali TZ and Epstein JI. False Positive Labeling of Prostate Cancer With High Molecular Weight Cytokeratin: p63 a more Specific Immunomarker for Basal Cells. Am J Surg Pathol 2008;32:1890-95.