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A comparative study of p40 and 34βe12 as basal cell markers in the diagnosis of prostate glandular proliferations

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Abstract: *Objective:* The immunohistochemical markers like 34βE12 (HMWCK) and p40 both of which are basal cell markers of prostate provide objective information to aid in diagnosis of specific lesion within the prostate. Our Objectives are a) To compare p40 and 34βE12 staining in the three groups of benign, atypical and malignant proliferations. b) To evaluate the staining pattern of the above markers in ASAP and hence its value in further classifying them into benign, malignant and borderline malignant categories. *Methods:* 81 prostatic samples were studied in the categories of benign, ASAP and malignant prostatic proliferations. Our study was compared with the similar several studies in the world literature with the similar sample size. *Results:* The sensitivity of both p40 and 34βE12 is 95.92%, specificity being 100%, positive predictive value being 100% and the negative predictive value being 94.12% suggesting a reasonably good comparison with each other. *Conclusion:* The use of p40 in the diagnoses of suspicious prostate glands and compares favourably and has close correlation between staining with 34βE12 in basal cells. Both of these tests complement each other and each of these markers provide a specific subset of information.

Keywords: Prostate Cancer, ASAP, p40, 34βE12.

Introduction

Prostate cancer is the most common form of cancer in men and is the second leading cause of death worldwide [1]. The diagnosis of prostate cancer is mainly done by combination of prostate specific antigen (PSA) levels, histological findings including architecture, nuclear features, ancillary features and basal cell loss as a hallmark of malignancy, cytological studies and immunohistochemical studies [2]. The basal cells represent the reserve cell compartment within the prostatic epithelium and the interruption and loss of the basal cell layer leads to genesis of invasive carcinoma from the precursor lesion [3].

High Grade Prostatic Intraepithelial Neoplasia (HGPIN) and Atypical Small Acinar Proliferation (ASAP) have a greater risk for subsequent cancer development. Two such markers are of interest are 34βE12 (HMWCK) and p40 both of which are basal cell markers. Our study compares the utility of p40 and 34βE12 IHC in the diagnosis of atypical glandular proliferations and proliferations suspicious of malignancy in prostate biopsies. Although morphological assessment is the primary tool, IHC should be considered as an aid to the final diagnoses. In our study percentage of positivity, staining pattern and staining intensity in all the 3 categories like benign, ASAP and malignant were compared between the HMWCK and p40 IHC markers.

Basal cell IHC may be used to reduce the number of inconclusive results [4] and the need of repeat biopsy thus preventing the biopsy related complications of hematuria, rectal bleeding, infections and acute urinary retension [5]. Repeat biopsies lead to delay in the diagnosis and treatment [6].

HMWCK, the antikeratin antibody 34βE12 also known as keratin 903 is the standard basal cell marker of the prostate gland [7]. HMWCK is the immunohistochemical staining by antibody against 34βE12 cytokeratin. The expression of 34βE12/ cytokeratin is considered positive if there is cytoplasmic positivity of basal cells. The staining can be continuous or discontinuous [1].

p40 is the isoform of p63 which is the basal cell marker of prostate cancer. The p63 gene, a p53 homologue, is located on chromosome 3q27-29 [8]. p63 has several isoforms with two major groups being TAp63 and ΔNp63. These isoforms differ in the structure of the N-terminal domain. TAp63 isoform contains a transactivation – competent 'TA' domain with homology to p53, which regulates expression of the growth-inhibitory genes. The ΔNp63 isoform contains an alternative transcriptionally–inactive 'ΔN' domain, which is thought to antagonize the activity of TAp63 and p53. So 'p63' is a 'two-in-one' family of opposing molecules: TA is a p53 like tumor suppressor and ΔN –is an oncogene.

Material and Methods

This prospective and retrospective study was carried out on all the prostate biopsies and TURP (transurethral resection of prostate) specimens submitted to the Department of Histopathology, Apollo Hospitals Jubilee Hills, Hyderabad during three years from January 2014 to October 2016. It was a Cross-sectional/observational study.

The study mainly included H&E staining and Immunohistochemical staining by p40 and HMWCK. Routine H & E staining was done using LEICA-ST5020 automated stainer.

Antibodies used for immunohistochemistry were:

- *p40:* PathnSitu ® rabbit polyclonal antibody. Catalogue number: HAP123
- *HMWCK:* Dako® monoclonal mouse anti-human cytokeratin HMWCK, clone-34βE12 (ready to use) Dako Autostainer. Catalogue number: 10110176.
- The IHC staining was done using Ventana Benchmark® XT automated IHC slide staining system.

Statistical formula: This was not applicable in the present study which is an observational and not a randomised control one.

Results

The sensitivity of both HMWCK and p40 is 95.92%, specificity being 100%, positive predictive value being 100% and the negative predictive value being 94.12%

Fig-1: Benign prostatic hyperplasia (HE, 40x)

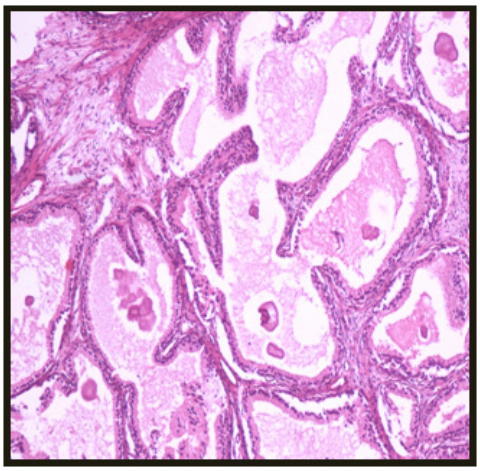
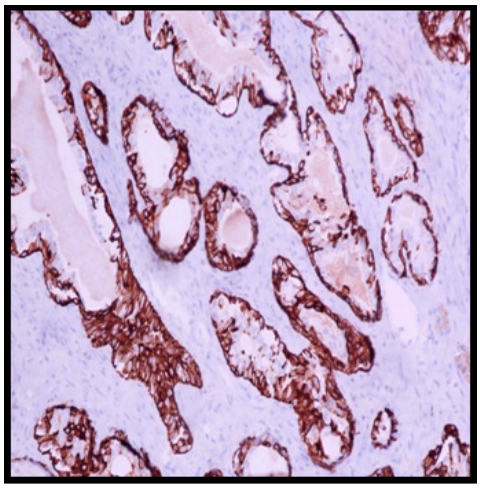
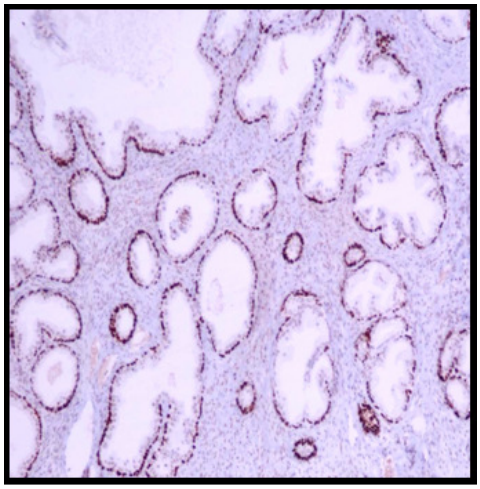


Fig-2: Benign prostatic hyperplasia (HMWCK IHC, 40x) (p40 IHC, 40x)



HMWCK IHC, 40x



p40 IHC, 40x

Fig-3: ASAP-malignant (HE, 20x)

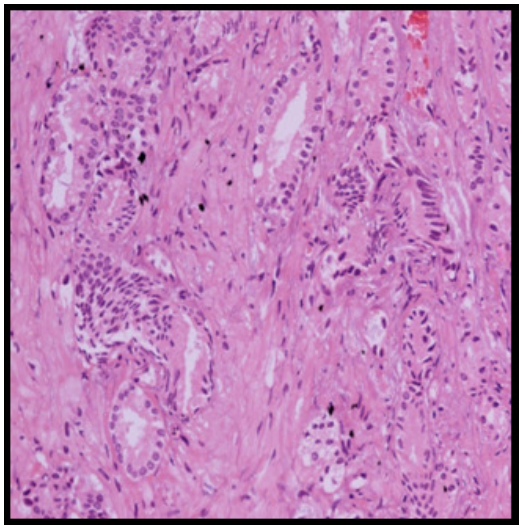
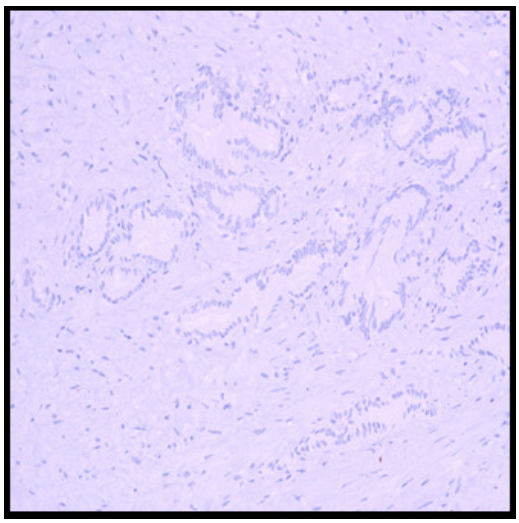
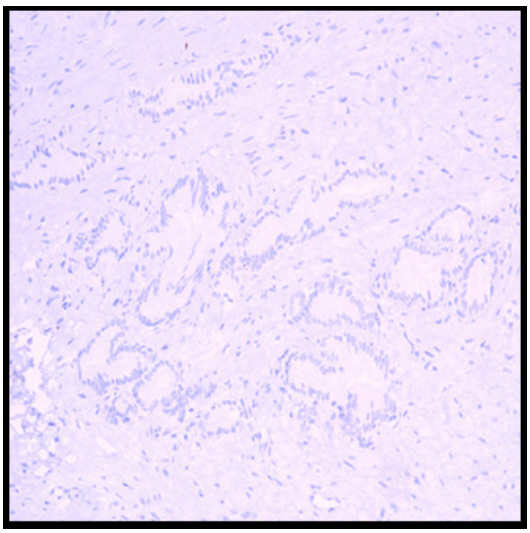


Fig-4: ASAP – Malignant (HMWCK IHC, 20x) (p40 IHC, 20x)



HMWCK IHC, 20x



p40 IHC, 20x

Fig-5: Adenocarcinoma Gleason score 10 (5+5), grade group V (HE, 20x)

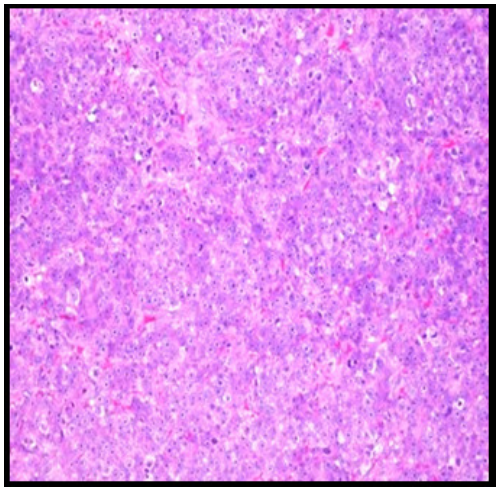
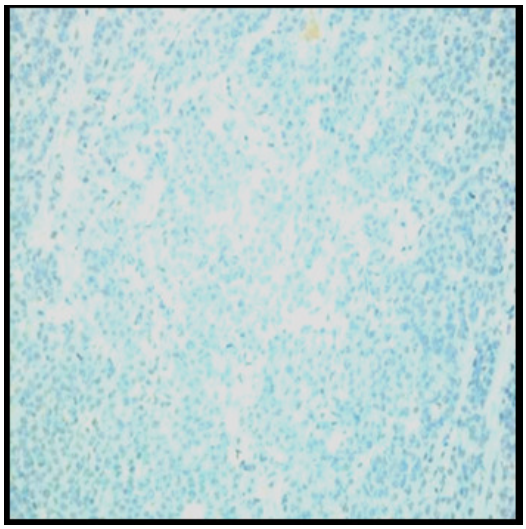
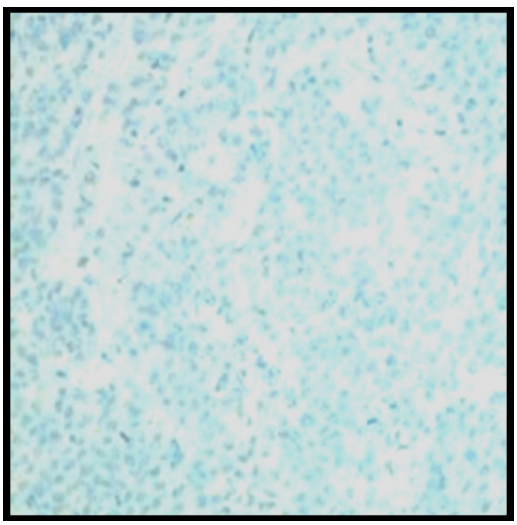


Fig-6: Adenocarcinoma Gleason score 10(5+5), grade group V (HMWCK, 20x) (p40, 20x)



HMWCK IHC, 20x



p40 IHC, 20x

Comparison between HMWCK and p40 in benign cases: The total score for 32 benign cases was analysed with 3 parameters including percentage positivity score, staining pattern and staining intensity between the HMWCK and p40 IHC by the Wilcoxon Signed Rank test and obtained a p value of 0.366 which was statistically insignificant.

Table-1: Comparison between HMWCK and p40 in benign cases					
Score	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
HMWCK	32	7.3125	1.255632	4	8
P40	32	7.40625	0.945597	5	8
p value: 0.366(Wilcoxon Signed Rank test) (statistically insignificant)					

Comparison between HMWCK and p40 in ASAP cases: The total score for 25 ASAP cases was analysed with 3 parameters including percentage positivity score, staining pattern and staining intensity between the HMWCK and p40 IHC by the Wilcoxon Signed Rank test and obtained a p value of 0.059 which was statistically insignificant.

Table-2: Comparison between HMWCK and p40 in ASAP cases					
Score	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
HMWCK	25	1.44	2.399305	0	6
P40	25	1.64	2.706166	0	7
p value: 0.059(Wilcoxon Signed Rank) (statistically insignificant)					

Comparison between HMWCK and p40 in HGPIN cases: The total score for 6 malignant cases associated with HGPIN was analysed with 3 parameters including percentage positivity score, staining pattern and staining intensity between the HMWCK and p40 IHC by the Wilcoxon Signed Ranks test and obtained a p value of 0.317 which was statistically insignificant.

Table-3: Comparison between HMWCK and p40 in HGPIN cases					
Score	**N**	**Mean**	**Std. Deviation**	**Minimum**	**Maximum**
HMWCK	6	7.83	0.408	7	8
P40	6	8	0	8	8
p value: 0.317(Wilcoxon Signed Rank) (statistically insignificant)					

Discussion

Prostate cancer remains one of the most common malignancies diagnosed among men [9]. The diagnosis of prostate cancer is done by use of traditional histological parameters, including architecture, nuclear features and ancillary feature. In the recent years the use of basal cell markers are used as adjuvant to morphology in diagnostically challenging cases with a very high sensitivity and specificity.

The basal cell markers are employed to study glandular proliferation of the prostatic tissue when present in small amounts and appear "atypical" in morphology. The differential diagnosis for these cases encompass lobular or partial atrophy, post-atrophic hyperplasia, adenosis, normal structures like verumontanum glands and inflammation associated changes [7]. There are several caveats associated with the use of basal cell markers for the diagnosis of prostatic adenocarcinoma.

The present study is a retrospective and prospective study during a period of 2014-2016 at the Department of Histopathology, Apollo Hospital, Jubilee Hills, Hyderabad.

The study included 81 cases of prostatic lesions, which were evaluated for p40 and HMWCK expression by using IHC.

Correlation of HMWCK expression with HE diagnoses: In our study we analyzed the association between HMWCK and HE diagnosis using a non parametric test namely Kruskal Wallis test. We report a highly significant association between HMWCK and HE diagnoses: the p value for this association in our study was less than 0.001.

Correlation of p40 expression with HE diagnoses: In our study we analyzed the association between p40 and HE diagnosis using a non parametric test namely Kruskal Wallis test. We report a highly significant association between p40 and HE diagnoses: the p value for this association in our study was less than 0.001.

Comparison between HMWCK and p40:

- The sensitivity of both HMWCK and p40 is 95.92%, specificity being 100%, positive predictive value being 100% and the negative predictive value being 94.12%
- Similarly both the markers were compared using Non parametric tests like Wilcoxon Signed Ranks test which showed that there was no statistical difference between staining for HMWCK and p40 (p= 0.627).
- A study by Hermann Brustman in 2015 also showed that there was no statistical difference between staining patterns for HMWCK and p40 (p=1.000, chi square test) [1].
- The use of HMWCK and p40 in a focus with only a few atypical glands is not diagnostic, since benign glands may not show uniform positivity with these markers. Negative staining for both these markers is most diagnostic when more than a few glands are present for evaluation and the morphologic features are very suspicious for carcinoma. So these markers are used to verify a suspicious focus of cancer rather than used to establish a diagnoses of cancer.

Conclusion

Our study supports for the use of p40 in the diagnoses of suspicious prostate glands and compares favourably and has close correlation between staining with HMWCK in basal cells. The percentage of positivity, staining pattern and staining intensity in all the 3 categories like benign, ASAP and malignant were comparable between the HMWCK and p40 IHC markers.

Acknowledgement

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