IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 16, Issue 3 Ver. IX (March. 2017), PP 22-28
www.iosrjournals.org

Diagnostic Utility of Immunohistochemical Markers in Prostate Cancer

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Abstract
Introduction: *Prostate cancer is a multifaceted disease comprises distinct biological subtypes with varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications. Over the last few decades there have been outstanding advances in prostate cancer management leading to earlier detection of disease. Prostatic cancer diagnosis is based on a combination of architectural, cytological and ancillary features.*
Immunohistochemistry (IHC) is a valuable adjunctive in diagnosis of minute foci of prostatic carcinoma and to differentiate it from benign mimickers & precursor lesions. Prostate cancer is now the 5th most common cancer in the world (in terms of number of new cases), and 2nd most common cancer in men.
Aims of study :1) To assess the diagnostic utility of IHC markers in morphologically ambiguous prostate carcinomas 2) To assess the efficacy of P63 over HMWCK 3) To know the level of diagnostic error in reporting of Prostatic lesions on routine H&E.
Materials &Methods : The Present study is a prospective study carried for a period of two years from June 2011 to May 2013 in Department of Pathology, Rangaraya medical college / Government general hospital, Kakinada. We received 310 cases of prostate biopsies in our department in the two year period. Out of the 310 cases, 40 cases were reported as suspicious of malignancy on H&E and are subjected for IHC.
Results &Analysis : 40 suspicious cases were subjected for IHC. Out of 40 cases, in only 20 cases (50%) there is disparity between diagnosis on H&E and diagnosis on IHC. In 20 cases, 14 cases (35%) were under-diagnosed as benign or pre-malignant or suspicious of malignancy. These got upgraded to HGPIN or carcinoma or carcinoma with foci of HGPIN after IHC study. Remaining 6 cases (15%) are downgraded from pre-malignant lesions to benign lesions.
Discussion : Our results are almost correlated with other studies.
Conclusion : 1.Even though diagnosis of prostate cancer is mainly based on architectural pattern, cytological and ancillary features, Immunohistochemistry acts as a most valuable adjunctive, which significantly increases the diagnostic accuracy in prostatic carcinoma.
2.Among basal cell markers, p63 has more sensitivity and specificity than HMWCK.
3.No single marker is having 100% sensitivity or specificity in diagnosis of prostatic carcinoma. Hence combination of a Basal cell marker (p63) with AMACR is more informative in the diagnosis of prostatic carcinoma.
Keywords: *Prostate carcinoma, Alpha methyl Co-A racemase (AMACR), Basal cell markers – HMWCK, P63.*

I. Introduction

Prostate cancer is a multifaceted disease comprises of distinct biological subtypes with diverse natural history, presenting a varied spectrum of clinical, pathologic and molecular features with different prognostic and therapeutic implications. Over the last few decades there have been outstanding advances in prostate cancer management leading to earlier detection of disease.

Prostatic cancer diagnosis is based on a combination of architectural, cytological and ancillary features. Immunohistochemistry (IHC) is a valuable adjunctive in diagnosis of minute foci of prostatic carcinoma and to differentiate it from benign mimickers & precursor lesions. Prostate cancer is now the 5th most common cancer in the world (in terms of number of new cases), and 2nd most common cancer in men.

Three IHC markers have been proved useful in the diagnosis ambiguous cases of Prostate Carcinoma. (Flow chart 1):

• α-methyl-acyl-coenzyme A racemase (AMACR), a positive diagnostic tissue biomarker of prostate cancer,

• Lack of staining with basal cell markers (34βE12-HMWCK & p63) supports the diagnosis of carcinoma in ambiguous cases.

II. Materials & Methods

The present study is a prospective study carried for a period of two years from June 2011 to May 2013 in Department of Pathology, Rangaraya medical college / Government general hospital, Kakinada. We received 310 cases of prostate biopsies in our department in the two year period. Out of the 310 cases, 226 cases were benign, 44 cases were frank malignancies and 40 are suspicious / premalignant cases.All the blocks and H&E stained slides of 40 suspicious cases were collected. For the TURP specimens, suspected chips were marked on the slide and were separated from the block and re-embedded, are subjected to IHC. For open prostatectomy specimens, one block with suspicious foci is selected and subjected to IHC. For core needle biopsies entire core is subjected to IHC.The cases with suspicious foci were subjected to immunohistochemistry (IHC) using HMWCK (34bE12), P63 and AMACR (p504S) markers. To act as controls 11 cases of proven carcinomas of various grades and 5 benign cases were taken.The 40 suspicious cases consists 14 open prostatectomies, 1 core needle biopsy and 25 were TURP chips. The age group of patients in present study is ranged from 50 to 95yrs.

IHC staining of biopsies were performed in Department of Pathology, GGH, Kakinada and these were divided into various categories like BPH, Basal cell hyperplasia, Cribriform hyperplasia, AAH, ASAP, HGPIN, Carcinoma.

III. Results

Out of 40 cases selected to IHC, in only 20 cases (50%) there is disparity between diagnosis on H&E(Table 1&2) and diagnosis on IHC. In 20 cases, 14 cases (35%) were under-diagnosed as benign or pre-malignant or suspicious of malignancy. These got upgraded to HGPIN or carcinoma or carcinoma with foci of HGPIN. Remaining 6 cases (15%) are downgraded from pre-malignant lesions to benign lesions.IHC was performed on the 40 suspicious cases using two basal cell markers HMWCK & P63 and a positive marker for carcinoma AMACR. After IHC staining results interpretated as circumferential & strong diffuse positivity with AMACR & negative stains with HMWCK & p63 – labelled as adenocarcinoma.Negative/ weak non circumferential AMACR stains & positive HMWCK &p63 in basal cells considered as adenosis/Atrophy.Positive staining with HMWCK & p63 in the luminal cells labelled as basal cell hyperplasia.

Suspicious foci with large glands exhibiting positive staining with both AMACR & HMWCK, P63 taken as HGPIN. Under these guidelines all 40cases subjected to IHC were interpreted Out of the 40 suspicious cases 14 cases (35%) are under-diagnosed (FIGURE 1 & 2) in which 9 (22.5%) cases were carcinomas, 2 cases were carcinoma with foci of HGPIN (5%) and 3 cases (7.5%) were HGPIN. Out of the 6 cases (15%) which got downgraded on IHC (FIGURE 3), 3 cases (7.5%) were basal cell hyperplasias, 2 cases (5%) were AAH and 1 case (2.5%) was BPH. Finally out of 40 suspicious cases, 26 cases (65%) were benign and 14 cases (35%) were diagnosed as malignant or premalignant lesions on application of IHC markers (Table 3 & 4).Out of the 40 suspicious cases, in 31 cases there is good correlation in staining of basal cells by P63 and HMWCK. In rest of the 9 cases, show superior basal cell layer staining by p63 when compared to HMWCK.

IV. Discussion

In 2001, Jiang et al examined AMACR expression in 137 prostatic carcinomas and 70 benign prostate cases by IHC using rabbit monoclonal antibody of AMACR. All 137 cases (100%) showed strong positivity irrespective of their Gleasons grade. In addition 88% of benign lesions show complete negativity for AMACR, with focal weak positivity in 12% of cases[1]. This is correlated with our study, where there is 100% positivity of Prostatic cancer controls (11/11) and 20% of benign controls (1/5) show focal weak positivity with AMACR.

According to Zhong Jiang et al, using AMACR as a positive marker alone might be misleading because weak expression of AMACR might be seen in benign glands, and expression of AMACR is seen in HGPIN and AAH. To avoid such errors, in our study along with AMACR we used basal cell markers also, which will enhance the diagnostic accuracy.[1]

Zhou et al demonstrated that, of 115 prostate biopsies diagnosed as atypical lesions by an expert pathologist, 34 (30%) were changed to a final diagnosis of cancer based on a positive AMACR immunostain Molinie et al were able to resolve 89% of 104 "ASAP" cases in needle biopsies using p63/AMACR antibody cocktail compared with only 53% with CK 5/6. [2]

Kunju et al were able to resolve 27 (93%) of 29 atypical biopsies after immunostaining with AMACR and basal cell marker.[3] In our study diagnosis of 40 suspicious cases (100%) are resolved using two basal cell

markers (P63 & HMWCK) and AMACR (Table 5). M.H. Weinstein et al proved that p63 is more superior in demonstrating Prostatic Basal Cells when compared to HMWCK[4] which correlated with our study. Our results reveal high expression of p63 (40 of 40, 100%) in normal basal cells and confirm the superiority over HMWCK (31 of 40, 77.5%), with an improvement in specificity.Beach et al found 82% AMACR expression in prostate cancer whatever may be the degree of differentiation i.e. Gleason score or morphological types. In our study controls we got 100% positivity in all grades and variants of prostatic carcinoma. In one case of foamy cell prostatic carcinoma there is focal expression of AMACR, but it is dark and circumferential staining, so interpreted as positive. In one case of high grade prostatic carcinoma, there is focal but dark staining of cytoplasm of the cancer cells and hence interpreted as positive. These results are also in agreement with the study of Jiang et al, who found numerous benign hypertrophic hyperplastic nodules negative for AMACR staining, indicating that cancer development is restricted to a subset of hypertrophic lesions which are premalignant or malignant, which may be identified by the enhanced expression of AMACR.[5]

Yang and colleagues found that AMACR was focally expressed in 10% of cases and diffusely positive in only 7.5% of cases of AAH.[7] In our study 1 out of 8 AAH cases (12.5% of AAH cases) showed focal expression of AMACR. In our study out of 40 suspicious cases, 26 were benign on IHC, of which 5 cases (12.5%) showed weak, focal, discontinuous positivity which were interpreted as negative.In 2003, Kunju et al directly compared the two antibodies and found that 68% of benign glands showed weak expression of AMACR with polyclonal antibody compared to 7% using the monoclonal antibody P504S (AMACR).This has correlated with our study as we used rabbit monoclonal antibody which is more sensitive of all as said by Kunju et al.[8]

IV. Summary

The basic architectural and cytological features can help only to some extent, the valuable adjunctive in these cases is IHC.

The two broad indications for use of immunohistochemistry in prostatic pathology:
(1) In confirming prostate carcinoma and/or distinguishing it from its many benign mimics,
(2) In distinguishing prostate carcinoma from non-prostatic malignancies that secondarily involve the prostate.

Prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP) traditionally have been used to confirm a prostatic tumor origin; however, they are not expressed uniformly in poorly differentiated Prostatic Carcinoma and might be negative in up to 27% and 19% of cases, respectively. They are mainly used to differentiate primary prostatic adenocarcinomas from metastatic adenocarcinomas. So, we conclude that in conjunction with morphology and clinical scenario, a combination of a basal cell marker (P63) and AMACR is of great value in combating the morphologically suspicious cases and thus significantly increasing the diagnostic accuracy in prostate cancer.

VI. Conclusion

1. Even though diagnosis of prostate cancer is mainly based on architectural pattern, cytological and ancillary features, Immunohistochemistry acts as a most valuable adjunctive, which significantly increases the diagnostic accuracy in prostatic carcinoma.
2. Among basal cell markers, p63 has more sensitivity and specificity than HMWCK.
3. No single marker is having 100% sensitivity or specificity in diagnosis of prostatic carcinoma. Hence a combination of a Basal cell marker (p63) with AMACR is more informative in the diagnosis of prostatic carcinoma.

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Flow Chart 1: Protocol of IHC markers interpretation

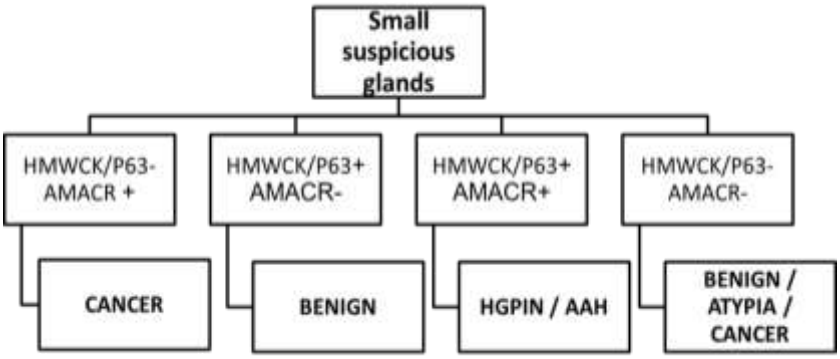

Table 1 : Diagnosis of total cases on H&E staining

Category	**No. of cases**	**Percentage (%)**
Benign	226	72.9%
Suspicious & pre-malignant	40	12.9%
Malignant	44	14.2%
Total	**310**	**100%**

Table 2 : Diagnosis of suspicious cases on H&E staining

Type of lesion	**No. of cases**	**Percentage**
Cribriform hyperplasia With ? PIN	2	5%
ASAP	5	12.5%
ASAP with ? PIN	6	15%
AAH	4	10%
AAH with ?PIN	7	17.5%
AAH with metaplasia	2	5%
Basal cell hyperplasia	2	5%
PIN changes	9	22.5%
?Carcinoma	3	7.5%
TOTAL	40	100%

Table 3 : Cases that are Underdiagnosed on H&E

Diagnosis on H&E	**No. of the cases**	**Diagnosis on IHC**
Basal cell hyperplasia	1	HGPIN
Cribriform hyperplasia	1	HGPIN
AAH	1	HGPIN
ASAP with PIN	2	HGPIN with carcinoma
ASAP	1	Carcinoma
ASAP with PIN	2	Carcinoma
AAH with PIN	1	Carcinoma
? Carcinoma	3	Carcinoma
PIN	2	Carcinoma
TOTAL	14	

Table 4 : Cases That Are Downgraded On IHC

Diagnosis on H&E	**No. of the cases**	**Diagnosis on IHC**
AAH with PIN	1	BPH
PIN changes	3	Basal cell hyperplasia
PIN changes	2	AAH
TOTAL	6	

Table 5 : Comparison With Other Studies

Study	**No. of cases**	**Markers used**	**No. of cases resolved**	**Percentage (%)**
Browne et al	123	AMACR	86	70%
Molinie et al	104	p63/AMACR antibody cocktail	93	89%
Kunju et al	29	AMACR and basal cell markers	27	93%
Kumarsen et al[9]	50	AMACR & HMWCK	49	98%
Our study	40	AMACR, HMWCK & P63	40	100%

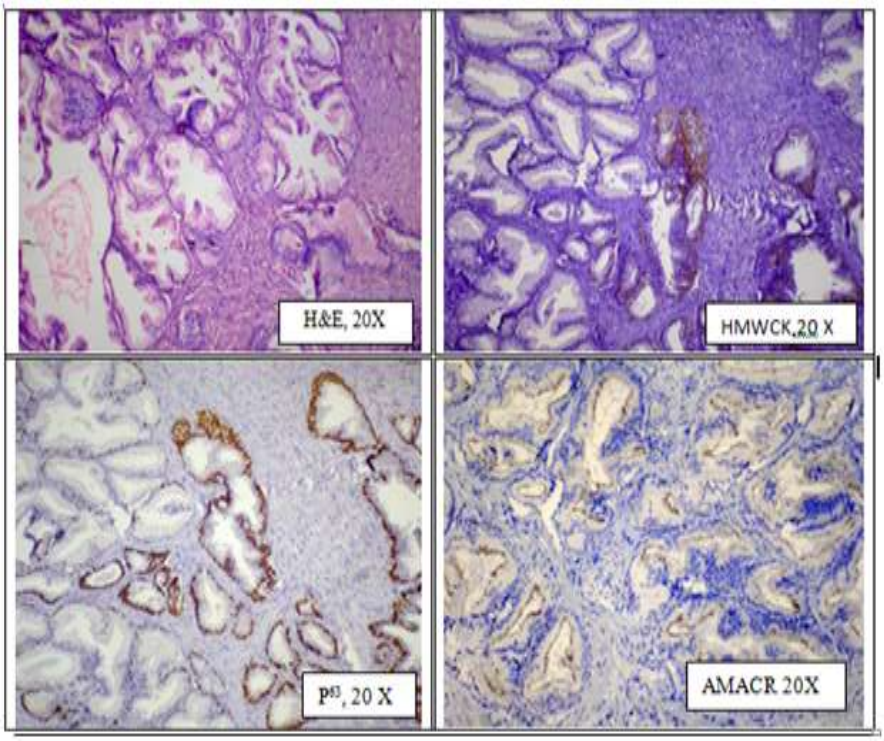

Figure 1: An under diagnosed case – **Biopsy no.464/12**
Diagnosis on H&E:PIN changes;**Final diagnosis on IHC**:Carcinoma with HGPIN
IHC : HMWCK & P63 –ve (in crowded glands); AMACR +ve(in all glands)

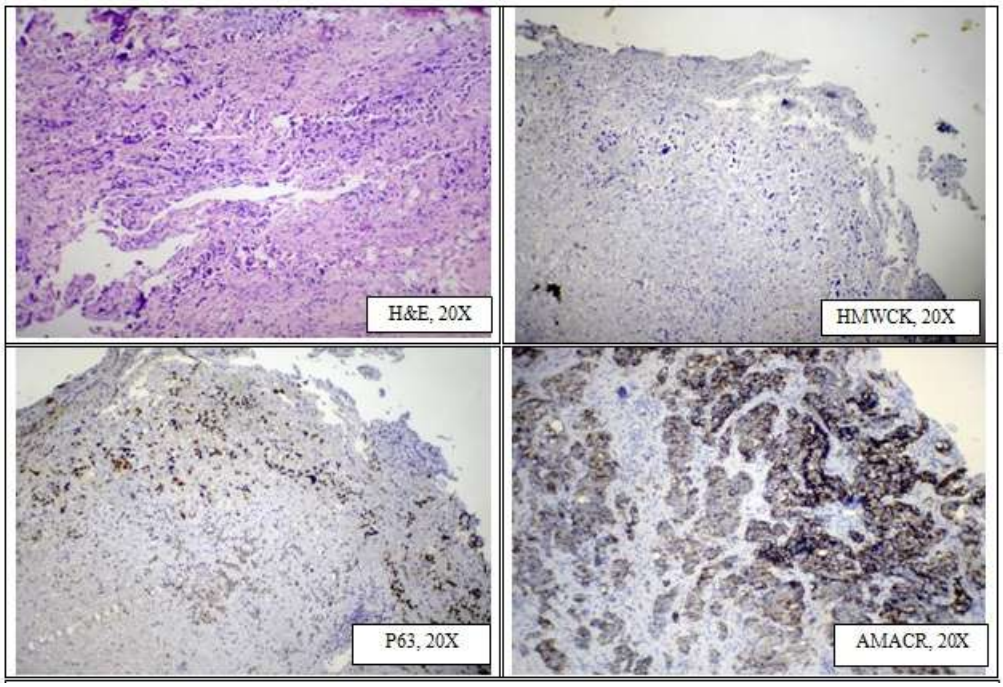

Figure 2 : An Under diagnosed case **– Biopsy no.1982/13.**
Diagnosis on H&E : Suspicious of carcinoma**; Final diagnosis on IHC :** Carcinoma**.**
IHC : HMWCK –ve; P63 Focally +ve; AMACR +ve diffusely

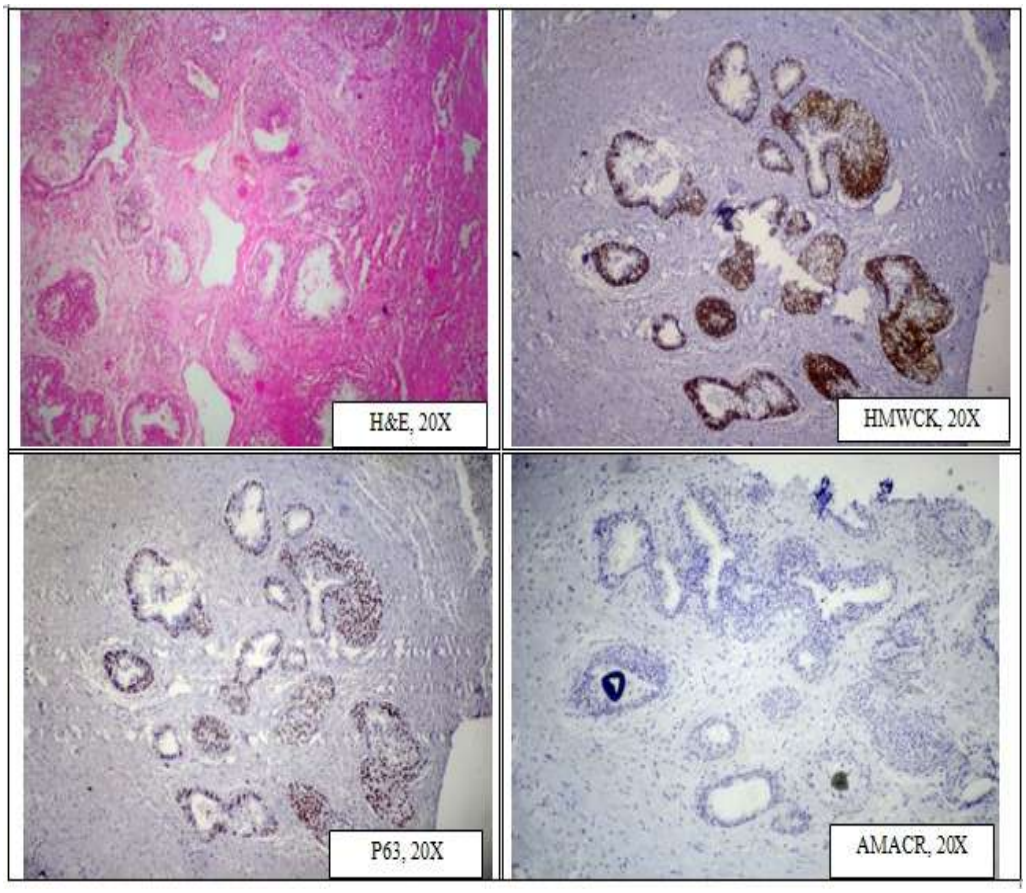

Figure 3 : A Downgraded Case - **Biopsy 2639/11**;
Diagnosis On H&E : HGPIN ; **Final Diagnosis Based On IHC** : Basal Cell Hyperplasia
IHC:HMWCK +ve; P 63 +ve; AMACR -ve.

List Of Abbreviations Used

AAH	Atypical Adenomatous Hyperplasia
AMACR	Alpha Methylacyl Co-A Racemase
ASAP	Atypical Small Acinar cell Proliferations
BPH	Benign Prostatic Hyperplasia
DAB	Diaminobenzidine tetrahydrochloride
HGPIN	High Grade Prostatic Intraepithelial Neoplasia
HMWCK	High Molecular Weight CytoKeratin (34βE12)
H&E	Hematoxylin & Eosin
IHC	Immunohistochemistry
TURP	Trans Urethral Resection of Prostate.
WHO	World Health Organization