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RESEARCH ARTICLE
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EVALUATE DIAGNOSTIC ACCURACY OF NKX3.1 WITH AMACR & P63 IN PROSTATIC ADENOCARCINOMA

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ABSTRACT

The most prevalent type of cancer in males is prostate adenocarcinoma. Based only on histologic features, it can occasionally be challenging to distinguish prostatic adenocarcinoma from prostatic mimickers. Using immunohistochemistry (IHC) and a negative immunological response to p63, along with positive staining for Alpha Methyl Acyl CoA Racemase (AMACR), cancer is diagnosed. The prostatic tumour suppressor gene NKX3.1 is found on chromosome 8p. While most primary prostatic adenocarcinomas stain positive for the NKX3.1 protein, most high-grade prostate cancers have it downregulated, and most metastatic prostate cancers have it completely lost (e.g., in 65% to 78% of lesions), according to most studies. Therefore, the study aimed to evaluate the diagnostic accuracy of Nkx3.1 in combination with AMACR & P63 in prostatic adenocarcinoma. This was an observational, prospective & retrospective study involving 56 samples of biopsies and specimens, conducted over a period of 12 months after the fulfillment of the criteria. After following the standard protocol for specimen staining, the IHC was carried out. The correlated histopathological features in all the cases were selected, and the diagnostic utility was determined statistically. The data was collected and analysed using the Chi-square test, all the results showed 5% level of significance & 95% confidence interval with a p-value of <0.05. The maximum age group of the patients was within 70-80 years, with the maximum cases of prostatic adenocarcinoma diagnosed in 42 (75%) patients, with 15 (26.8%) in grade 4. A high statistical significance was seen between the association of diagnosis with IHC expression. To distinguish between adenocarcinoma and its mimics of prostatic lesions, NKX3.1 as a diagnostic performance in a combination with p63 and AMACR proves to be significant. In suspected situations, IHC is advised to minimise diagnostic error.

Keywords: NKX3.1, AMACR, P63, prostatic adenocarcinoma, cancer, immunohistochemistry, tumour, diagnosis

INTRODUCTION

One of the most prevalent malignancies in males is Prostate Adenocarcinoma.[1] The Caribbean, Australia, North America, Southern Africa, and Western and Northern Europe have the largest populations.[2] In Asia, the number of older males using screening methods such as digital rectal examination, prostate-specific antigen (PSA), and ultrasound (USG) has been rising.[3] Serum PSA levels, digital rectal examination, and biopsy samples taken from resected tissues (TURP chips) or TRUS-guided needle biopsies are used to diagnose it.[4]

Several foci of adenocarcinoma have been observed in prostatic needle biopsy specimens since the early diagnosis of prostatic cancer through widespread screening of males, and the presence of several benign mimickers has made histology extremely difficult.[5] Some benign mimics of adenocarcinoma, such as basal cell hyperplasia, squamous metaplasia, low- and high-grade prostatic intraepithelial neoplasia, atypical adenomatous hyperplasia, or the presence of normal anatomic structures, including seminal vesicles and Cowper's gland, can make the diagnosis challenging. These factors can lead to inappropriate treatment, psychological effects, and medical-legal repercussions.[6-8] Sometimes histologic features alone are insufficient to diagnose prostate mimickers. In a few cases, a diagnosis is possible based on the presence/absence of basal cells. The basal cell layer is absent in prostatic adenocarcinoma, whereas it is present in mimickers. The main drawback of employing solely negative markers is the patchy existence of basal cells within the benign glands.[9,10] Because p63 immunostaining may not always reveal positivity in the basal cells, it may occasionally produce negative results, which does not always rule out benign glands. Similarly, AMACR is particularly helpful when combined with p63 because a negative result does not always mean that the sample is malignant.[5]

Alpha-methylacyl-CoA racemase (AMACR), an enzyme marker specifically expressed in prostate cancer, and prostatic mimickers may be detected using an IHC marker such as p63.[11] A positive AMACR result and a negative p63 result are used to diagnose prostatic cancer. Cases of well-differentiated adenocarcinoma were used as the control for AMACR.[12-14] Since histomorphology alone was insufficient to confirm the mimickers, p63, a basal cell marker, has been used in conjunction with AMACR to distinguish between mimickers and adenocarcinoma.[15]

The expression of the androgen-regulated homeodomain gene NKX3.1 is mostly seen in the prostate epithelium.[16] NKX3.1 is found on chromosome 8p21.2, which exhibits loss of heterozygosity (LOH) in 35% to 86% of prostatic adenocarcinomas and 12–89% of high-grade prostatic intraepithelial neoplasia (PIN). As the grade and stage of advanced prostate cancer grow, so does the incidence of LOH on chromosome 8p. Protein secretion, epithelial cell differentiation, proliferation, and prostate branching morphogenesis are all impaired in mice when NKX3.1 is specifically disrupted.[17] Prostate epithelial hyperplasia and PIN have also been observed in animals lacking NKX3.1, and deletion of one or both NKX3.1 alleles causes faster and more aggressive prostate carcinogenesis in mice having targeted mutation of Pten or Cdkn1b (encoding p27).[18] Since the remaining genotype of NKX3.1 has not been shown to contain any mutations, it seems to be a haploinsufficient tumour suppressor gene.[19] Therefore, in this study, we evaluated the diagnostic accuracy of NKX3.1 using AMACR & P63 in prostatic adenocarcinoma.

METHODOLOGY

The study was an observational, prospective, and retrospective study that involved 56 biopsies & specimens received in the pathology laboratory. The study was conducted at the Bharati Vidyapeeth Deemed University, Medical College, Pune, with inclusion criteria that included all prostatic adenocarcinomas and exclusion criteria for non-malignant conditions, including BPH, Bacterial Prostatitis, and Chronic Prostatitis. The study duration was 12 months. The tools used for data collection were the test request forms and the histopathological records. There were no anticipated risk factors involved in the study.

After being stained with H&E dye, all 56 cases were further analysed and divided into three groups: prostatic adenocarcinoma, mimics, and suspected of malignancy. After that, IHC using p63 and AMACR was performed on every patient in each group.

Clean glass slides coated with poly-L-lysine were used to capture sections for the immunohistochemical test. Applying 0.3% hydrogen peroxide in methanol inhibited endogenous peroxidase activity, and epitope retrieval was carried out according to the manufacturer's instructions. A monoclonal anti-p63 antibody and an anti-AMACR antibody were used for IHC. For p63, normal breast tissue served as a control, and for AMACR, kidney tissue from the proximal tubules was used. Positive or negative results were interpreted from the IHC for p63. If the prostate cancer showed circumferential or cytoplasmic finely granular staining, IHC with AMACR was considered positive. Nuclear positivity with p63 in the basal cell layer was also regarded as positive staining.
Statistical analysis was performed using SPSS (version 25). Sensitivity and specificity were calculated using the Chi-square test, along with the p-value.

RESULTS

Out of 56 samples, the distribution of patients according to diagnosis included 10 (17.9%) with atypical small acinar proliferation, while prostatic adenocarcinoma was diagnosed in 42 (75%) patients & Suspicious adenocarcinoma was diagnosed in the remaining 4 (7.1%) patients, respectively. The majority of patients belonged to the 70-80-year age group. At the same time, there were 14 (25%) patients in the group grade of 0. Group Grade 1 had only a single patient, while Group Grade 2 saw 11 (19.6%) patients. There were a total of 7 (12.5%), 15 (26.8%), & 8 (14.3%) patients with Grade 3, Grade 4 & Grade 5, respectively. [Table 1]

Table 1: Frequency distribution of group grade of the patients according to the adenocarcinoma

Group Grade	Frequency (N)	Percentage (%)
0	14	25.0
1	01	1.8
2	11	19.6
3	07	12.5
4	15	26.8
5	08	14.3
Total	**56**	**100.0**

The results showed that p63 was positive in 40% of patients diagnosed with Atypical small acinar proliferation and in 25% of patients diagnosed with Suspicious for adenocarcinoma, respectively. It was negative for 60% of patients and 75% of patients diagnosed with Atypical small acinar and Suspicious for adenocarcinoma, respectively. This finding was statistically highly significant ($p < 0.001$). AMACR was positive in 60% of patients diagnosed with Atypical small acinar, 76.20% of patients diagnosed with prostatic adenocarcinoma, and in 50% of patients diagnosed with Suspicious for adenocarcinoma, respectively. This difference was not statistically significant ($p > 0.05$). NKX31 was positive in 20% of patients diagnosed with Atypical small acinar, 100% of patients diagnosed with prostatic adenocarcinoma, and in 75% of patients diagnosed with Suspicious for adenocarcinoma, respectively. This finding was statistically highly significant ($p < 0.001$).

Table 2: Association of Diagnosis with IHC (immunohistochemistry)expression

Marker	Result	Atypical small acinar proliferation	Prostatic adenocarcinoma	Suspicious for adenocarcinoma	Total	Chi-Square	p-value
p63	Negative	6 (60.0%)	42 (100.0%)	3 (75.0%)	51 (91.1%)	17.26	<0.001
	Positive	4 (40.0%)	0 (0.0%)	1 (25.0%)	5 (8.9%)		
AMACR	Negative	4 (40.0%)	10 (23.8%)	2 (50.0%)	16 (28.6%)	2.01	0.367
	Positive	6 (60.0%)	32 (76.2%)	2 (50.0%)	40 (71.4%)		
NKX3.1	Negative	8 (80.0%)	0 (0.0%)	1 (25.0%)	9 (16.1%)	38.58	<0.001
	Positive	2 (20.0%)	42 (100.0%)	3 (75.0%)	47 (83.9%)		
Total		**10 (100%)**	**42 (100%)**	**4 (100%)**	**56 (100%)**		

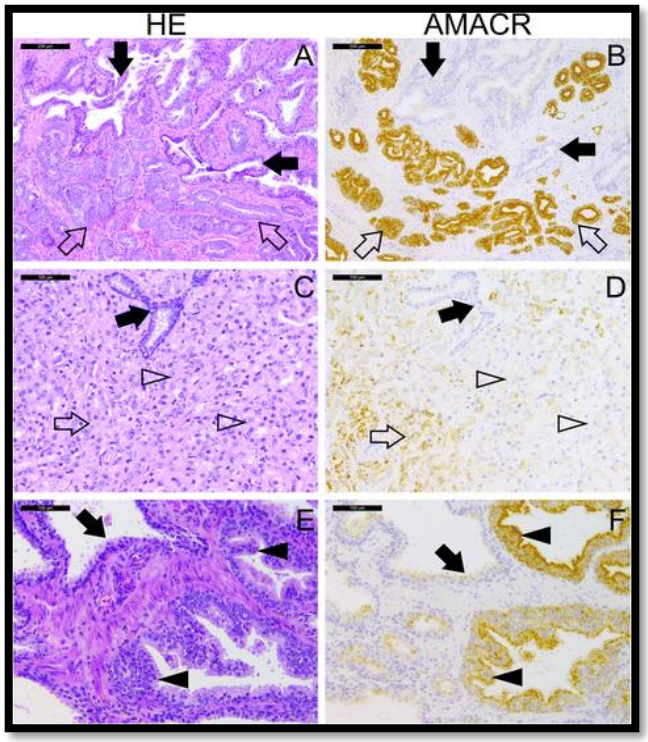

Figure 1: Immunohistochemical analysis of AMACR protein expression in different prostatic lesions. Hematoxylin-eosin (HE) and AMACR staining from the same region of interest are shown. a, b Typical acinar Gleason 3 + 4 adenocarcinoma stains strongly for AMACR (open arrow) while the normal glands are AMACR negative (filled arrow). c, d Gleason 4 + 5 adenocarcinoma shows focal positive staining for AMACR (open arrow) while some of the poorly differentiated carcinoma glands (open arrowhead) and normal glands (filled arrow) are AMACR negative. e, f: High-grade PIN lesion shows positive AMACR staining (filled arrowhead) while morphologically normal glands show weak or no AMACR staining (filled arrow). Scale bar 200 µm (a-b), 100 µm (c-f).

DISCUSSION

The frequency of prostate needle biopsies conducted has significantly increased due to the widespread use of prostate-specific antigen as a cancer serum marker. The diagnostic procedure has been challenged, though, because it has been linked to an increase in inflammation and other prostate abnormalities.[20] Even if the tiny foci of atypical glands are seen after biopsy, it may be difficult to diagnose prostate cancer. Numerous mimics of prostate lesions, such as seminal vesicle, low- and high-grade prostatic intraepithelial neoplasia, squamous metaplasia, basal cell hyperplasia, and atrophy, can also make it challenging. To diagnose prostatic mimickers, IHC has recently been used in conjunction with morphology and monoclonal antibodies targeting basal cell markers, such as p63 and AMACR, for prostatic adenocarcinoma. This tool has improved the diagnostic precision of prostate adenocarcinoma worldwide, leading to better treatment options.[21]

According to several research studies and publications conducted globally, the AMACR immunostain is effective in detecting prostatic adenocarcinoma. As such, it is a very significant, diagnostic, and practical IHC marker for prostate cancer diagnosis. According to a few studies, high-grade prostatic intraepithelial neoplasia also shows AMACR expression. Combining AMACR with basal cell markers, such as p63, has been shown to increase its sensitivity and specificity.[22] In the work done by M. Patel, it was observed that both p63 & AMACR antibodies, which are markers for both benign and malignant conditions, were used to demonstrate how these markers are expressed in distinguishing between prostatic mimics and adenocarcinoma.[17,23,24]

In their analysis of p63 expression in several instances, Sadeghifar et al. found that p63 protein expression was very diffusely positive in basal cells and hyperplastic prostate glands, but it was

patchy in prostate atrophy, along with high-grade prostate intraepithelial neoplasia. Furthermore, it was proposed that High Molecular Weight Cytokeratin and p63 were detected as negative in malignant prostate lesions but positive in all 60 patient cases of benign lesions.[25] Jiang and colleagues identified prostate carcinomas using a triple-antibody cocktail consisting of AMACR, HMWCK 34βE12, and p63. The results demonstrated a particular and sensitive marker. Therefore, it was determined that AMACR should be employed in an antibody cocktail containing a basal cell marker for diagnostic evaluation in suspected prostate tissue lesions.[7]

Bowen et al. found a correlation between the progression of prostate cancer and the loss of NKX3.1 protein expression as determined by IHC. Specifically, they found that 20% of high-grade PIN, 6% of stage T1a/b samples, 22% of stage T3/4 samples, 34% of hormone-refractory prostate cancers, and 78% of metastases had total loss of NKX3.1 staining.[26] In contrast, Korkmaz et al. performed in situ hybridisation for mRNA expression and immunohistochemistry (IHC) for protein staining on adjacent TMA slides. They found that there was no association between NKX3.1 mRNA or protein expression and tumour grade or clinical stage, and that a large proportion of all prostatic adenocarcinoma cases were positive for both the mRNA and the protein.[27] Prostatic adenocarcinomas were shown to express the NKX3.1 protein by IHC in 66% of initial untreated tumors, 44% of untreated metastatic tumors & 27.3% of castrate-resistant/hormone-refractory tumors, according to Gelmann et al.[28]

Immunohistochemical studies have been used in two primary areas where it is essential to determine the prostatic origin of a neoplasm: transurethral resection or biopsy specimens, where the most common scenario is the need to differentiate poorly distinguished high-grade primary prostatic adenocarcinoma compared to high-grade urothelial carcinoma as well as metastatic adenocarcinomas of unknown origin, where the differential diagnosis is much wider. PSA and PSAP may occasionally be expressed at least slightly in a variety of non-prostate tumours, although they are expressed at a much lower level in metastatic prostate carcinomas.[8]

CONCLUSION

In situations with difficult morphology, immunohistochemistry employing p63 and AMACR is very helpful in distinguishing well-differentiated adenocarcinoma from prostatic mimickers. To reduce the effects of under- and overtreating patients, they are therefore used to determine the precise kind of disease in challenging tiny prostate samples taken by core-needle biopsy. Combining the recent studies, it was found that most poorly differentiated primary prostate cancers had strong NKX3.1 staining. If used in the correct clinicopathologic context, adding NKX3.1 protein staining to a panel of markers may aid in diagnosing metastatic lesions with an unclear primary origin.

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