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Optimizing Detection of Prostate Cancer by AMACR and P63 on Prostatic Needle Biopsy-Sudanese Experience

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Abstract: ***Background and Objective: Establishing a definitive diagnosis is the major challenge in prostatic needle biopsyespecially in small focus of cancer as there is no single specific histologic feature for malignancy or due to the presence of benign mimickers.However, the lack of specific marker for prostate cancer, which can be utilized with routine H&E to enhance diagnostic precision remains a limitation.Recently, basal cell marker P63 and prostate cancer marker AMACR have been used as adjuvant to morphology for diagnosis of such challenging foci.This study was set out to determine the sensitivity and specificity of these markers and their usefulness in differentiation between prostate adenocarcinoma and benign prostatic hyperplasia (BPH) on prostatic needle biopsy. Method: A 250 archival prostate needle biopsies during 2017–2019 were selected and sorted according to routine H&E as follow; 100 prostatic adenocarcinoma, 50 Atypical (ATYP) and 100 of BPH.Sections of 5 µm thickness were cut, then Immunohistochemistry (IHC) was performed using monoclonal AMACR and P63 antibodies and the results were analyzed using SPSS. Results: AMACR had 95% sensitivity and 98% specificity, statistically AMACR wassignificantly expressed in prostate adenocarcinoma P.value (0.000). P63 had 95% sensitivity and 95% specificity, it was significantly expressed in benign prostatic hyperplasia P. value (0.000). By IHC 38/50 atypical foci (ATYP), was confirmed as adenocarcinoma while 12/50 case reported as BPH. The study concluded that combination of AMACR as a positive marker and p63 as a negative marker will improve sensitivity, specificity and enhances diagnostic precision.***

Keywords: Prostate cancer, TRUS biopsy, Sudan. AMACR, P63

1. Introduction

Prostate cancer is the second common cancer and sixth leading cause of deaths in men worldwide. Histologically 95% of prostatic cancers are adenocarcinomas (Lalit, *et al*, 2019).At 2018, from 10218 new cancer cases in Sudanese men, 938 (9.2%) cases were diagnosed as prostate cancer with prevalence rate (6.53%)(Ferlay 2012).Clinically prostate cancer progression is relatively slow and may be asymptomatic (Kumar *et al*, 2010). Currently diagnosis of prostate cancer depends on suspicious findings in either digital rectal examination (DRE) or elevated serum PSA then followed by more sophisticated diagnostic techniques such as transrectal ultrasound (TRUS) and guided biopsy (Lalit *et al*, 2019).Cancer detection in prostate biopsy is often challenging, especially when the cancer focus is minute. It needs a methodical approach using a constellation for architectural and cytological features of cancer glands.Sometimes may requires immunohistochemistry (IHC) (Rajal and Zhou, 2012). The use of immunohistochemistry markers individually or with two or three markers combined in a panel to establish the diagnosis of carcinoma in a morphologically atypical small focus of prostate glands is currently a common laboratory practice. Immunostaining for basal cell markers are typically used in a "negative" diagnostic mode, to show absence of basal cells in prostate cancer, sole reliance on such markers is not advocated, and the identification of a combination of major and minor histologic features of prostate cancer is crucial for achieving clinical diagnostic accuracy. AMACR, and p63 in combination offer a great value in ensuring the absence of a basal layer with positive AMACR labeling in such small foci. Alpha-methyl-acyl-CoA-racemase (AMACR), is an enzyme involved in beta-oxidation of fatty acids (branched chain) and their derivatives, it is up regulated in prostate cancer. A monoclonal antibody to AMACR, known as P504S has been produced and is currently commercially available for use on formalin-fixed, paraffin embedded tissue sections (Biswas and Talukdar, 2019). However there are varied reports regarding the expression of AMACR in prostate cancer which ranges from 62% to 100%.

The p53 homolog p63 encodes for different isotypes that can either transactivate p53 reporter genes (TAp63) or act as p53-dominant negatives (ΔNp63), and p63 is expressed in the basal or myoepithelial cells of many epithelial organs. The p63 expression in the prostate gland is restricted to basal cells and is absent in secretory and neuroendocrine cells and ΔNp63α isotype is the most abundantly represented isotype in normal prostate basal cells. P63 gene is essential for normal stem cell function in the prostate, it believed to play a critical role in the regulation of growth and development of a variety of epithelial organs, including the prostate gland (Arthi, 2019).

2. Methodology

Case selection

The prostatic biopsies were done according to the European Association of Urology recommendations in the

Departments of Urology by consultant Urologist at different hospitals and Centers in Khartoum State and sent in 10% buffered formalin fixative filled container to Histopathology Department at El-rahmma Diagnostic Center. The laboratory diagnosis of prostatic needle biopsy was done by experienced histopathologist using hematoxylin and eosin (H&E) stain. By reviewing the medical records, clinical and laboratory information of all cases are collected (Serum tPSA, age and diagnosis). A 250 archival paraffin wax embedded blocks of prostate needle biopsies were selected for this study and sorted as follow;100 blocks of prostatic adenocarcinoma and 50 blocks of Atypical glands suspicious for cancer (ATYP) as cases. For control purpose 100 blocks of benign prostatic hyperplasia were selected.

Immunohistochemistry for AMACR and p63.

According to the Envision kit (DAKO Corp., Carpinteria, CA) AMACR was stained. Firstly slides deparaffinized, hydrated and then treated by citrate buffer (pH 6.0) and steamed for 14 min. For blocking of endogenous peroxidase activity, slides incubated with DAKO peroxidase block for 5 min at room temperature then washed, and incubated with primary antibody (1:16,000 dilution of antiserum) overnight at 4°C. Secondary antirabbit antibody-coated polymer peroxidase complex was added for 30 min. Substrate/ chromogen (DAB) was added and incubated for 10 min. Slides are counterstained with Mayer's hematoxylin for 2 min. For double labeling of AMACR and p63, the anti-p63 mouse monoclonal antibody cocktail (1:100 dilution; Lab Vision Corp., Fremont, CA) was added after the anti-racemese antibody incubation and incubated for 45 min at room temperature. The secondary antirabbit and antimouse HRP conjugates were sequentially added, and the reaction was developed as above. Finally slides mounted using DPX.

Evaluation of IHC

Immunostaining for p63 will interpreted as positive/ negative. Positive staining was defined as positive staining of nuclei of basal cells. Positive staining was taken as evidence of benignity and negative staining of an entire suspicious focus was taken as presumptive evidence of malignancy. AMACR results were considered positive, in case of circumferential, dark, diffuse or granular, cytoplasmic or luminal staining. IHC results will considered as negative; if there was an absence of staining or if only focal weak non-circumferential fine granular staining was seen with the absence of staining in the adjacent benign glands. The brownish cytoplasmic AMACR stain for different sections will evaluated for intensity of stain and proportion of carcinoma cells stained using intensity score stated by Warrick, *et al*. Intensity score was rated 0 (non circumferential staining), 1+ (focal apical granular staining), 2+ (diffuse weak cytoplasmic staining), or 3+ (strong, cytoplasmic and luminal staining). Proportion was rated with respect to percentage of positively stained cells, as follows: 0 (<5%cells stained), 1+ (5% to 25% of cells stained), 2+ (26% to 50% of cell stained), 3+ (51% to 75% of cells stained),and 4+ (76% to 100% of cells stained). The intensity and proportion scores were added to give an overall score, with 7 being the highest possible. All scores>0 were considered AMACR positive (Warrick *et al*, 2013).

Data analysis

Data will be analyzed using IBM SPSS advanced statistics version 22 (SPSS Inc., Chicago, IL). Numerical data expressed as mean and standard deviation or median and range as appropriate. Qualitative data expressed as frequency and percentage. Chi-square test is used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between three groups were done using nonparametric ANOVA. The sensitivity and specificity were calculated using receiver operating characteristic (ROC) curve and the area under the curve (AUC). P-value <0.05 is considered significant for all tests.

3. Results

Table 1: AMACR expression in Study groups

AMACR expression	Study groups			Total
	Prostatic Adenocarcinoma (cases)	Benign Prostatic Hyperplasia (control)	Atypical Foci Suspicious for Cancer (cases)	
Positive	95	2	38	135
Negative	5	98	12	115
Total	100	100	50	250

Table 2: The area under the curve (ROC) curve for AMACR according to H&E

Variable	AMACR
Classification variable	H & E
Sample size	200
Positive group [a]	100(50.00%)
Negative group [b]	100(50.00%)
Area under the ROC curve (AUC)	0.965
Significance level P (Area=0.5)	<0.0001
Sensitivity	95.00
Specificity	98.00

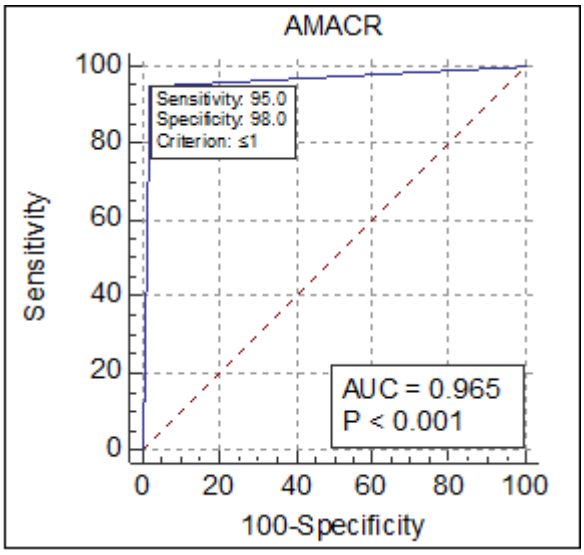

Figure 1: The Sensitivity and Specificity ofAMACR according to H&E

Table 3: P63 expression in study groups

P63 expression	Study groups			Total
	Prostatic Adenocarcinoma (cases)	Benign Prostatic Hyperplasia (control)	Atypical Foci Suspicious for Cancer (cases)	
Positive	5	97	14	116
Negative	95	3	36	134
Total	100	100	50	250

Table 4: The area under the curve (ROC) curve for P63 according to H&E

Variable	P63
Classification variable	H & E
Sample size	200
Positive group [a]	100(50.00%)
Negative group [b]	100(50.00%)
Area under the ROC curve (AUC)	0.950
Significance level P (Area=0.5)	<0.0001
Sensitivity	95.00
Specificity	95.00

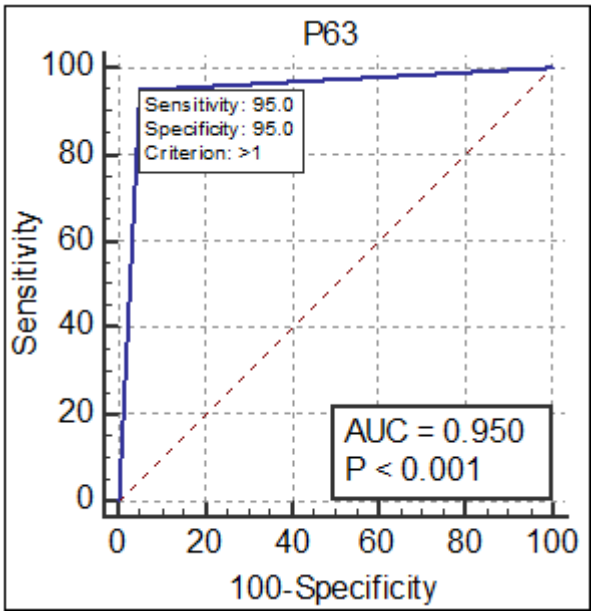

Figure 2: ROC curve for P63 according to H&E

Table 5: Expression of Amacr and P63 in atypical foci suspicious for cancer:

	Amacr	P63
Positive	38	14
Negative	12	36
Total	50	50

4. Discussion

The detection of prostate cancer in needle biopsies is mainly based on a constellation of morphologic features but frequently may be extremely challenging, especially when small localized focus suggestive for cancer is noted in the specimen. However the definitive feature of prostate cancer is the loss of basal cells. So in such challenging foci, basal cell markers are used as an adjunct to verify and support the diagnosis (Angela and Lakshmi, 2013).

Regarding IHC results in this study, out of 100 prostate adenocarcinoma cases confirmed by routine H&E, 95 (95%) showed positive AMACR staining Table (1). AMACR was significantly expressed in prostate adenocarcinoma (P = 0.000) as correlated with benign prostatic hyperplasia. The sensitivity of AMACR is 95% and specificity is 98%. This finding was in agreement with Arthi and Dhanalakshmi, 2019 who reported that only two out of 19 cases categorized as prostatic carcinoma showed negative cytoplasmic staining of AMACR. (Rathod, *et al*, 2019) also found that the sensitivity and specificity of AMACR was 90% and 100% respectively with high expression in prostate cancer as compared with benign lesions of prostate (P < 0.001). AMACR is a highly specific marker for diagnosis of prostate cancer with positive predictive value (PPV) 97.9%, this result agree with Biswas and Talukdar, 2019 who found that positive predictive value of AMACR was 96.77%. Fatima *et al*, 2019 on 10 cases of prostate adenocarcinoma reported that the sensitivity of AMACR was 100% and specificity was 95.4%.

In present study 5 (5%) cases of prostate adenocarcinoma showed negative AMACR staining, this finding is supported by Biswas and Talukdar, 2019 study which reported that 11.7% of prostate adenocarcinoma cases were negative for AMACR staining using monoclonal anti AMACR antibody thus recommended careful examination of morphologic pattern and combination of AMACR with basal cell marker for exclusion of prostate cancer in prostate needle biopsy specimen. Rashed, *et al*, 2012 reported that some variants of prostatic adenocarcinoma can be AMACR negative, this variants include atrophic, foamy gland and pseudo hyperplastic. Srivastava*et al*, 2019 evaluated AMACR expression in 30 cases of prostatic adenocarcinoma using polyclonal anti AMACR antibody and reported that all the 30 cases showed positive AMACR staining with 100% sensitivity. Difference in AMACR sensitivity can be a result of using different antibodies (polyclonal and monoclonal) for detection of prostate cancer. Other factors like concentration of the primary antibody, staining technique (manual or automated) and antigen retrieval protocol can affect AMACR sensitivity.

In this study AMACR was positive in two BPH cases Table (1) similar to Fatima SK *et al*. 2019. A possible explanation was according to Evans *et al*, 2003 who reported that pseudo neoplasms also (atypical adenomatous hyperplasia, atrophy, post atrophic hyperplasia and basal cell metaplasia) shows positive AMACR immunoreactivity. Also Leav *et al*,2003 reported a phenomenon that called “Field effect” plays an important role in such positive BPH cases. Furthermore over staining phenomenon which was suggested by Yang *et al* 2002.

In this study, 89 (89%) of prostatic adenocarcinoma cases showed AMACR staining in more than 50% cells this result agreed with Shrivastava *et al*, 2019 who found 26 (86.7%) of prostatic adenocarcinoma showed AMACR proportion score +3 staining (more than 50% cells were stained).

The current study reported that P63 was significantly expressed in benign prostatic hyperplasia P. value (0.000). Of 100 BPH biopsies in the present study, 97 were positive for P63 immunostaining with 95% sensitivity and 95% specificity Table. This result was in agreement with study by

Premalatha *et al*.2019 which reported sensitivity of p63 was 93% and disagree with those by Rathod, *et al* 2019 and Al-Sayed Ibrahim *et al* 2019 that reported positive expression of p63 in all non-cancerous lesions (100%) this differences may be due to differences in staining methods.

The current study reported positive p63 staining in five prostate adenocarcinoma cases table (4.12), four cases in Gleason group 3 and one case in Gleason group 5 this finding was in agreement with Singh, *et al* who reported that, rarely high grade prostate cancers express p63. This finding usually is not a diagnostic problem, as AMACR is positive in the malignant cells and morphology is standard diagnostic tool for evaluation of malignancy. Premalatha *ET al*.2019 noted that staining of p63 may indicates an altered and potentially oncogenic function of the missed localized protein in the tumour progression and survival. It is associated with highertumor grade and increased mortality.

Of the 50 atypical foci suspicious for cancer cases table (5), there was a change of diagnosis based on morphology, and staining with p63 and AMACR in 38 (76%) cases from suspicious for cancer to Adenocarcinoma, 36 of which (36/38) expressed positive immunostaining for Amacr and negative for p63 while (2/38) were expressed positive Immunostaining for both Amacr and p63. While another 12 (24%) case which was 'Suspicious for malignancy', were reported as benign prostatic hyperplasia after immunostaining. Fatima *et al* 2019 reported change in diagnosis from atypical foci suspicious for cancer to adenocarcinoma in four of six cases (66%). The reasons for the error in the provisional diagnosis may be either due to limited focus of cancer with very few malignant acini or due to difficulties of benign mimickers.

5. Conclusion

Routine H&E is the gold standard, Combination of AMACR as a positive marker and p63as as a negative marker, with a simple immunostaining procedure, on prostatic needle biopsy and small foci lesions will improve sensitivity, specificity and enhances diagnostic precision thus reducing the risk of false negatives especially in atypical suspicious lesions and also reducing the need for additional unnecessary biopsies. Finally the cost of immunohistochemistry staining techniques remains lower than a new series of biopsies.

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