Original Article

Evaluation of mucin histochemistry in benign and malignant prostatic lesion and their correlation PSA level

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ABSTRACT

Background: The major causes of prostatic enlargement are nodular hyperplasia, prostatitis and neoplasm including prostatic intraepithelial neoplasm. Acidic mucin is absent in benign prostatic glands and is present in some prostatic adenocarcinomas. Mucin stain may be an adjunctive aid in the diagnosis of prostatic adenocarcinoma. This study aims to find out role of acidic mucin in differentiating benign and malignant lesion together with the aid of PSA level.

Materials and Methods: A study was done from August 2006 to August 2008. Serum PSA level was performed. Biopsy specimen were processed and stained with Hematoxylin and Eosin as well as Alcian Blue and Periodic Acid Schiff. Data was coded and entered into SPSS v. 11 and Chi-square test and scatter plot was done to analyze the findings.

RESULTS: Out of 207 prostatic specimens studied, 78.3% showed BPH with or without prostatitis whereas 12% showed PIN and 8.7% malignancy. PSA density showed statistical significance with p= 0.02, in favor of malignancy. Alcian blue positivity was in 77.8% of malignant cases and 18.5 % in benign cases (p = 0.000003). Acid mucin was more in well differentiating tumours decreasing significantly in high grade malignancies. Combined diagnostic value of PSA>10 ng/ml and Alcian blue positivity showed increase in specificity to 96.8% for diagnosing a malignant lesion, with positive predictive value of 57.1%.

CONCLUSION: PSA density can be used to identify and pick up malignant cases where intermediate levels of PSA values are common. Alcian blue stain can help in assessing the degree of differentiation in malignant cases.

INTRODUCTION

Enlargement of prostate is one of the leading cause of morbidity in all men after the age of 40 years. The major causes are nodular hyperplasia, prostatitis and neoplasm

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including prostatic intraepithelial neoplasm. By far the most common is nodular hyperplasia of the prostate which can be seen in approximately 20% of men 40 years of age, a figure that increases to 70% by age 60 and to 90% by age 70.[1] Prostatitis is another important cause seeking medical attention. Internationally, there is an increased incidence of mycobacterial prostatitis, concomitant with disseminated disease, in underdeveloped countries.

The prostatic cancer is the second most common cause of cancer death in men and is the fourth most common male malignancy worldwide.[2] It is estimated that more than 300,000 new cases are detected anually of which approximately 41,000 prove to be fatal.[3] Prostatic intraepithelial neoplasia (PIN) has been identified as a precursor lesion to prostatic carcinoma.[4] PIN has a high predictive value as a marker for adenocarcinoma, and its identification warrants repeat biopsy for concurrent or subsequent invasive carcinoma.

Numerous reports have claimed that because acidic mucin is absent in benign prostatic glands and is present in some prostatic adenocarcinomas, this stain may be an adjunctive aid in the diagnosis of adenocarcinoma of the prostate.[5]

This study was under taken to find out role of mucin in differentiating benign and malignant lesion together with the aid of other investigations like PSA level and clinicoradiological correlates.

MATERIALS AND METHODS

This was an analytical study done from August 2006 to August 2008 of patients who underwent prostatic biopsies and surgeries due to various prostatic pathologies in Manipal Teaching Hospital. Biopsy specimen included were tru-cut biopsy, suprapubic prostatectomy, TURP specimen. Histopathological sections were stained by Hematoxylin and Eosin for microscopic features and special stains were done with Alcian Blue and Periodic Acid Schiff (PAS). Serum of these patients was collected for prostatic specific antigen (PSA) where possible. Relevant clinical data were collected. Data was coded and entered into SPSS (Software Programme For Statistical Study) version 11 and analysed. Chi-square test was used to calculate the significance for categorized variables. Scatter plot was done to see the distribution of PSA in different prostatic lesions.

RESULTS

A total of 207 cases of patients of prostatism were studied during the period of two years. The mean age of patients was 70.22±9.84, the range from 36 years to 97 years. Maximum cases were from age group 70-79 years constituting 42 % (n=87) of total cases followed by age group between 60-69 years 35.1 %(n=57).

The major histopathological divisions were done for Benign, PIN and malignant cases. A total of 162 cases of benign lesions, 27 of PIN and 18 of malignant cases were identified histopathologically. The age group distribution of the various prostatic lesions is depicted in the table 1.

Out of these, (n=72) 34.8% were found to have benign prostatic hyperplasia, 43.5% (n =90) benign prostatic hyperplasia with chronic prostatitis, 12 %(n=27) PIN and

8.7% (n= 18) malignancy.

Of the total PIN cases most of the cases were diagnosed as PIN - low grade (22/27) and among malignant cases 2 were well differentiated, 8 were moderately differentiated, 8 were poorly-differentiated adenocarcinomas.

Prostatic specific antigen and histopathology.

Prostatic specific antigen (PSA) was done in total of 63 patients out of which 45 were in benign conditions, 11 in malignant conditions and 7 in PIN cases. PSA distribution in different prostatic lesions is depicted in the following table 2.

When cut off of PSA value was taken as 10 ng /ml, 90.9% of malignancy showed PSA >10, and 44.5% of benign lesions still had PSA value > 10ng/ml, which was statistically significant in favor of malignancy, with p = 0.01 (chi-square of 5.92)

Table 1: Distribution of age group with major histological groups

Age Group	Malignant	Benign	PIN	Total
<50	1(5.6%)	4 (2.46%)	-	5
50-59	-	13(8%)	3(11.1%)	16
60-69	6(33.3%)	57(35.1%)	6(22.2%)	69
70-79	9(50%)	68(41.9%)	10(37.1%)	87
>80	2(11.1%)	20(12.3%)	8(29.6%)	30
Total	**18**	**162**	**27**	**207**

Table 2: PSA level versus histopathology

PSA Level (ng/ml)	Malignant	Benign	PIN	Total
<4	-	10	1	11
4- 10	1	15	3	19
10-20	1	12	1	14
20-50	5	7	2	14
>50	4	1	-	5
Total	11	45	7	63

Table 3: Diagnostic value of prostatic malignancy at various levels of PSA

Serum PSA(ng/ml)	Sensitivity	Specificity	Positive predictive value
>=4 to <10	100%	21.2%	21.2%
>=10 to <20	90.9%	55.8%	30.3%
>=20 to <50	81.8%	82%	53%
>=50	36.4%	98.1%	80%

Table 4: Correlation of PSA density with histopathology

PSA density (ng/ml/cm3)	Malignant	Benign	PIN	Total
<0.15	1(11.1%)	16(57.1%)	2(50%)	19
>0.15	8(88.9%)	12(42.9%)	2(50%)	22
Total	9	28	4	41

Table 5: Mucin stains in Prostatic lesions

Histopathology	PAS positive	Alcian Bleu positive	Positive predictive value
Carcinoma	8(44.4%)	14(77.8%)	21.2%
Benign	142(87.8%)	30(18.5%)	30.3%
PIN	24(88.8%)	5(18.5%)	53%

PAS negativity with malignancy $\chi 2 = 18.78$, df=1,p=0.00068
AB positivity with malignancy $\chi 2 = 30.03$, df=1,p=0.000003

While evaluating the sensitivity and specificity of particular cut offs, PSA >=50 ng/ml had specificity of 98% but had sensitivity of just 36%, whereas PSA >10 ng/ml had 55.8% specific and 90.9 % sensitive .When cut off value of PSA>20ng/ml was taken the sensitivity and specificity for picking up malignancy was both 82 %, so for our endogenous population the PSA value of more than 20ng/ml most likely can be taken for diagnosing malignant cases.(Table 3)

The PSA density was calculated in both the benign histological conditions and malignancy and shown in table 4. There was statistical significance of PSA density > 0.15 ng/ml/cm3 in malignancy as compared to benign lesions with p=0.02 (Fischer exact test)

Mucin Stains in Prostatic Pathologies:

PAS positivity and Alcian blue positivity in benign, PIN and carcinoma cases are showin in table 5. P value for PAS negativity in malignancy is 0.00068 which is statistically significant. P value for AB positivity in malignancy is 0.000003 which is again statistically significant for diagnosis of malignancy. Combined Diagnostic value of PSA>10ng/ml and Alcian Blue positivity in Malignancy:

Since PSA >10ng/ml was seen in majority of malignant cases and Alcian blue was seen in most of the malignant cases so combined diagnostic value of both of these together was analysed which showed high statistical significance with p <0.0001. Serum PSA and Alcian blue positive in malignancy has significant chi-square value ($\chi 2 =41.35$, df=1). It was also observed that Alcian blue positivity decreased with the decrease in differentiation showing positivity in only 50% of high grade prostatic carcinoma. In low grade and intermediate grade prostate cancers, however,

all were alcian blue stain positive.

DISCUSSION

During the study period, 207 patients with history of enlarged prostate were studied. Most of the cases were found out to be Nodular hyperplasia of Prostate with chronic prostatitis, followed by Nodular hyperplasia of prostate alone in 43.5% and 34.8% respectively , followed by PIN I and prostatic carcinoma in 10.6% and 8.7%

Result of the present study shows 90.9% of malignant lesion had PSA >10 ng/ml with sensitivity of 90.9% and specificity of 55.8%, When cut off of 50 ng/ml was taken it had specificity of 98% but had sensitivity of just 36% for malignancy.

In a study done by Arista-Nasr J et al[6] in Mexico median PSA values was 11.2ng/ml in benign cases and 45.6ng/ml in malignancy. There was a considerable overlap of PSA levels in benign and malignant cases and only values above 30ng/ml were highly suggestive of malignancy. A study performed by Gerstenbluth RE et. al[7] University School of Medicine, Ohio, USA, demonstrated that serum PSA > 50 ng/ml was 98.5% accurate in predicting the presence of prostatic carcinoma in tissue biopsy which was consistent with our finding of 98% specificity. In our study PSA was higher than 10 ng/ml almost 44.2% benign diseases and majority of these cases were Nodular hyperplasia of Prostate with prostatitis. In these cases the sensitivity would have been better if these cases of suspected prostatitis were treated with course of antibiotic and reevaluated for PSA value.

In chronic prostatitis various proposed mechanisms of elevation of PSA include leakage of PSA into the blood stream, hypervascularity, and altered vascular permeability secondary to inflammation as described by Sindhwani et al.[8] Evidence from the literature indicates that antimicrobial treatment may lower the PSA levels to what is considered the normal range. Despite that, general recommendations for the practical management are lacking and undetected prostate cancer in men with chronic prostatitis remains a difficult issue.[9]

In our study since most of the biopsies were having features of chronic prostatitis PSA was considerably high so screening with cut off of PSA>4 ng/ml would be misleading and will require lot of biopsy, hence PSA after treatment would be recommended and the PSA cut off for our own endogenous population will be important for screening program.

The concept of PSA density is based on the assumption that, as non-neoplastic prostate tissue does leak some PSA into the circulation, for PSA to be useful in diagnosing prostate

cancer, values should be corrected for total gland volume. Transrectal ultrasound is used to produce an estimate of gland volume and a threshold of 0.15 has been suggested as being discriminatory for cancer.[10]

In this study 88.9% of malignancy showed PSA density > 0.15 ng/ml/cm^3 which was statistically significant with p value of 0.02 in favor of malignancy. PSA density was a better predictor of prostate cancer in Chinese men with PSA levels of 4-10 ng/ml as shown by Zheng XY et al[11], especially those who have had prior ultrasound-determined measurements of prostate volume. PSA density was a significantly better indicator of prostate cancer than f/t PSA. The sensitivity and specificity of PSA density at a cutoff of 0.134 ng/ml/cm^3 was 90 %and 33.7%, respectively. Similar findings were observed in other studies.[12,13]

In benign lesions 87.8% cases were PAS positive and 18.5% were Alcian blue positive while in cases of carcinoma prostate Alcian blue positivity was seen in 77.8% of cases as compared 44.4 % which were PAS positive. This finding is because of the fact that acid mucosubstances are produced more by carcinoma than by benign prostates. Our findings are in consistent with previous studies done by Hukill and Vidone et al[14] who reported 80 % Alcian positivity in carcinoma prostate cases. Similar study done by Mathur SK et al[15] showed neutral mucin was more frequently seen in BPH 93.3% as compared to carcinoma 36% while acid mucin was found in more cases of carcinoma prostate 68 % as compared to benign prostatic lesion in 16%.They also observed that the positivity of Alcian blue was more in well differentiated tumor decreasing significantly in high grade malignancies. Luna –More S et al[16] studied 130 cases of prostatic carcinomas and 22 cases of atypical adenomatous hyperplasia (AAH) for neutral and acid mucins and found that incidence of acid mucin was higher in Prostatic carcinoma.

In our study we observed that alcian blue positivity was seen in 100% cases of low grade and intermediate grade prostatic carcinoma with Gleason score of 2-7 but the alcian blue positivity dropped to 50% in high grade prostatic carcinoma with Gleason score >8, showing that in poorly differentiated carcinoma alcian blue positivity is less. This finding was in consistent with study by Mathur et al and Taylor et al.[15,17] The other observation that was made in this study was combining the alcian blue positivity with PSA value cut off of >10 ng/ml, showed a high specificity for malignancy, 96.8% specific for malignancy.

CONCLUSION

Mucin stains like Alcian blue and PAS helps to identify malignant cases and specificity will be increased to 96.8% combining the Alcian blue positivity in cases of PSA >10ng/ml. PSA density may be helpful in differentiating nodular hyperplasia of prostate, prostatitis with carcinoma prostate.

A larger population based prospective study is required to find out the cutoff value for taking biopsy after a screening PSA for our endogenous population so that cases of prostatic carcinoma are identified in earlier stage and treatment can be started promptly.

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