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Original Research Article

Evaluation of mucin histochemistry in relation to p63 expression in nodular hyperplasia and adenocarcinoma of prostate

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ABSTRACT

Background: Prostate cancer is a leading cause of death in men. Nodular hyperplasia and adenocarcinoma are common causes of prostatic enlargement. Diagnosis of these lesions on routine biopsies can be difficult for pathologists. Immunohistochemical stain p63 can help, but it is costly and not widely available. The present study aimed to evaluate the usefulness of mucin histochemistry in relation to p63 expression in differentiating nodular hyperplasia and adenocarcinoma of prostate.
Methods: This study was conducted in the department of pathology at Dhaka medical college from July 2018 to June 2020. 50 cases of prostatic lesions (25 NHP and 25 adenocarcinoma) were examined using histomorphology. The sections were stained with periodic acid Schiff (PAS) to identify neutral mucins and Alcian blue (2.5 pH) to identify acidic mucins. Additionally, p63 antibody was used in immunohistochemistry.
Results: NHP showed positivity for neutral mucin (96% with PAS stain) but not for acidic mucin (Alcian blue stain), while prostatic carcinoma showed positivity for both neutral mucin (28%) and acidic mucin (44%). The grade group 1 tumors of prostatic carcinoma showed 100% positivity for acid mucin, with a decrease in Alcian blue staining as the grade increased. P63 was positive in 100% of NHP cases and negative in 100% of prostatic carcinoma cases.
Conclusions: Positivity for acidic mucins with Alcian blue stain can be a helpful diagnostic tool to differentiate well differentiated adenocarcinomas from benign lesions where facility for p63 immuno-stain is not available and poor people who cannot afford the cost of immunohistochemistry.

Keywords: NHP, Prostatic adenocarcinoma, Mucin histochemistry, p63

INTRODUCTION

Enlargement of the prostate is one of the leading causes of morbidity in men of older age. The most common causes of prostatic enlargement are nodular hyperplasia and prostatic adenocarcinoma. By far the most common is nodular hyperplasia of prostate (NHP).[1] The prevalence of NHP rises markedly with increased age. Autopsy studies revealed a histological prevalence of 8%, 50% and 90% in the 4th, 6th and 9th decades of life respectively.[2] Prostate cancer is the second most common cancer and the fifth leading cause of cancer related death

in men worldwide.[3] The incidence and mortality of prostate cancer is correlated with increasing age and it is diverse in different parts of the world.[4] In Bangladesh 2252 new cases were diagnosed in the year 2018 which causes 1.5% of all cancer related death.[5]

Confirmatory diagnosis and patient management of prostatic lesions depend on histopathological examination. But sometimes diagnosis of prostatic carcinoma on routine biopsies can be challenging when pathologists are faced with certain problems such as limited tissue sample, small foci of carcinoma or is the separation of well differentiated adenocarcinoma from vast number of benign or atypical small gland proliferations.[6,7] At present, immunohistochemistry becomes extremely helpful in the diagnosis of prostatic lesions. Basal cell marker p63 can be used to differentiate benign and malignant prostatic lesions.[8] But it is a complicated, costly procedure and also not available at all centres. For precise diagnosis of prostatic carcinoma, there is a need of a marker which is specific, cost effective and can be useful in peripheral areas where the advanced techniques are not available.

Different studies suggest that neutral mucin (detected by PAS) is more frequently observed in benign prostatic lesions, while acid mucin (detected by Alcian blue) is found more commonly in prostatic carcinoma. So, demonstration of mucin can play an important role to differentiate prostatic carcinoma from benign hyperplasia.

METHODS

This cross-sectional study was carried out at the department of pathology, Dhaka medical college, Dhaka from March 2018 to June 2020. Histo-morphologically diagnosed 25 NHP and 25 adenocarcinoma, total 50 cases of prostatic lesions were included in the study. Patients who had been previously treated or had a history of prostate cancer, or did not have sufficient tissue for immunostaining for p63 were excluded from the study. Anyone unwilling to participate were also excluded from the study. During the collection of specimens, all relevant information was recorded systematically in a predesigned data sheet. All the cases were numbered chronologically and the same number was given to histological, mucin stains as well as in immunohistochemical slides.

Hematoxylin and eosin-stained sections of each case were reviewed to confirm the histological diagnosis and modified Gleason grading was done in case of adenocarcinoma. Then according to Gleason grade group all malignant cases were categorized into grade group 1-5. Mucin histochemistry was done by PAS for neutral mucin and Alcian blue for acid mucin. Immunostaining for p63 were also done to demonstrate the presence or absence of basal cells. Basal cell staining was considered positive if strong to moderate brownish nuclear staining of basal cells and ≥25% of the glands were stained. [9]

RESULTS

Histo-morphologically diagnosed 25 nodular hyperplasia and 25 adenocarcinomas of prostate were included in this study. Age distribution of the patients ranged from 45 to 98 years. In case of NHP most of the patients 48% were found in between 61-70 years of age. Out of adenocarcinoma patients, maximum 36% patients age was between 71 to 80 years. The overall mean (±SD) age of the patients was 68.42 (±12.08) years. Statistically significant ($p<0.05$) difference was observed between the age groups of adenocarcinomas and NHP (Figure 1).

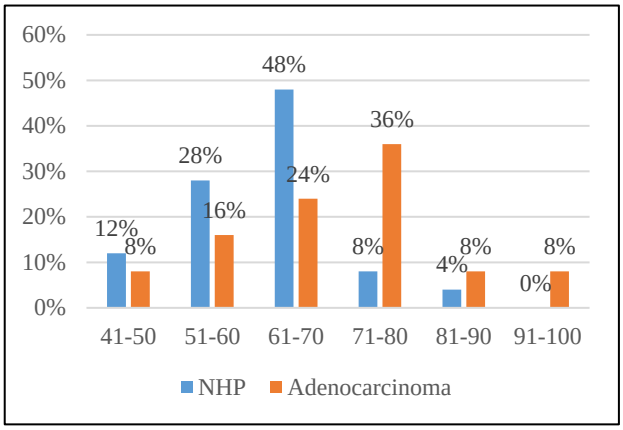

Figure 1: Age distribution of study population, (n=50).
P value=0.047^s, S=significant. Unpaired student's 't' test was done to measure the level of significance

Modified Gleason grading was done in case of adenocarcinoma. Out of 25 cases of adenocarcinoma, maximum 9 (36%) cases were in grade group 5 (Table 1).

Table 1: Distribution of study population according to Gleason grading and grade group of adenocarcinomas, (n=25).

Grade group	Gleason's score	Frequency	Percentages (%)
1	≤3+3=6	2	8
2	3+4=7	7	28
3	4+3=7	4	16
4	4+4=8 3+5=8	3	12
5	≥4+5=9	9	36
Total		25	100

PAS staining was done in all 50 cases to detect neutral mucin. Among NHP, 24 cases (96%) were PAS positive. Seven cases (28%) of adenocarcinoma were PAS positive. The difference was statistically significant ($p<0.5$) (Table 2).

Alcian blue staining was done to detect acid mucin in 50 cases of prostatic lesion. Alcian blue staining was positive in 11 (44%) cases of adenocarcinoma out of total 25 cases. Whereas, Alcian blue staining was negative in

all the cases (100%) of NHP. The difference was statistically significant (p<0.5)

Table 2: Distribution of study population according to PAS staining, (n=25).

PAS	Adeno-carcinoma		NHP		P value
	N	%	N	%	
Positive	7	28	24	96	<0.001
Negative	18	72	1	4	
Total	25	100	25	100	

*Chi square test was done to measure the level of significance.

Table 3: Distribution of study population according to Alcian blue staining, (n=25).

Alcian blue	Adeno-carcinoma		NHP		P value
	N	%	N	%	
Positive	11	44	0	0	<0.001
Negative	14	56	25	100	
Total	25	100	25	100	

s=significant, *Chi square test was done to measure the level of significance.

Figure 2 shows Alcian blue positivity in different grade group of adenocarcinomas. Maximum 100% cases were positive in grade group 1.

Immunostaining for p63 were done on all the 50 cases to detect the basal cell layer of prostatic glands. Twenty-five cases (100%) of NHP showed positive p63 expression and all 25 cases (100%) of adenocarcinoma showed negative expression of p63.

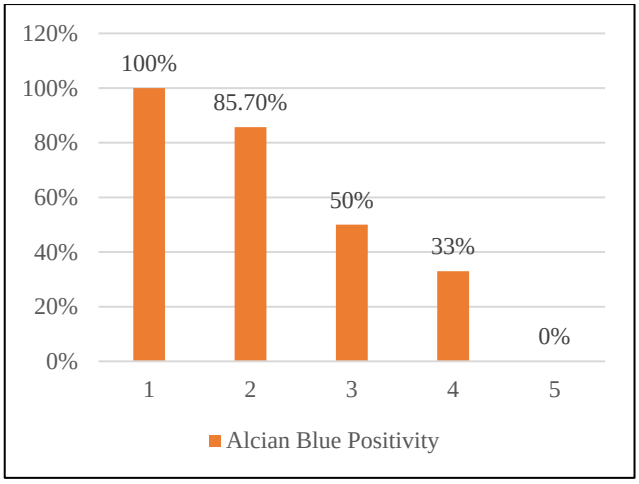

Figure 2: Alcian blue positivity in different grade group of prostatic adenocarcinomas, (n=25).

Table 4: Distribution of study population according to expression of p63, (n=50).

P63	NHP		Adenocarcinoma	
	N	%	N	%
Positive	25	100	0	0
Negative	0	0	25	100
Total	25	100	25	100

Considering mucin histochemistry and p63 expression in different grade group of adenocarcinomas, none of the cases showed PAS positivity in grade group 1 and 3. All cases were Alcian blue positive in grade group 1 followed by 85.7% in grade group 2. All (100.0%) cases showed negative expression of p63 in all grade group.

Table 5: Mucin histochemistry and p63 expression in different grade group of adenocarcinomas, (n=25).

Grade group	No. of cases	PAS positive		Alcian blue positive		P63 expression	
		N	%	N	%	N	%
1	2	0	0	2	100.0	0	0
2	7	5	71.4	6	85.7	0	0
3	4	0	0	2	50.0	0	0
4	3	1	14.3	1	33.3	0	0
5	9	1	14.3	0	0	0	0

DISCUSSION

NHP and adenocarcinoma are the two major causes of prostate enlargement. Treatment options and prognosis of these two lesions differ significantly, so they must be diagnosed accurately. For precise diagnosis of NHP and adenocarcinoma, mucin histochemistry can play an important role due to its simple procedure and cost effectiveness. The diagnosis of malignancy is often based on the absence of basal cells.[10] Therefore, basal cell marker (p63) can be useful in differentiating benign hyperplasia from prostatic adenocarcinoma.[8]

This cross-sectional study was undertaken to evaluate the role of mucin histochemistry in relation to p63 expression in NHP and Prostatic carcinoma. Histo-morphologically diagnosed 25 NHP and 25 adenocarcinoma, a total 50 prostatic lesions were included in this study. Mucin histochemistry was done to detect neutral mucin by PAS and acid mucin by Alcian blue stain. Expression of p63 was assessed by immunohistochemistry.

The incidence of benign and malignant lesions of prostate are strongly related to age. In this study it was observed that age distribution of the study patients ranged from 45 to 98 years. In NHP majority of cases (48%) were found

in between 61-70 years. The mean (±SD) age was 65.04 (±10.38) years with minimum and maximum values were 48 and 90 years respectively. Out of adenocarcinoma patients, maximum patients age was between 71 to 80 years. The mean (±SD) age was 71.80 (±12.91) years with minimum and maximum values were 45 and 98 years respectively. The overall mean (±SD) age of the patients was 68.42 (±12.08) years. The study result is almost similar to the study done by Agarwal et al in India. They reported the incidence of NHP was most commonly seen between the age group of 61-70 years and adenocarcinoma was above the age of 70 years.[11] In another study by Bastola and Talwar in Nepal found that the mean age of patients was 70.22±9.84. Maximum cases were from age group 70-79 years in both NHP (41%) and adenocarcinoma (50%).[12]

In current study, out of 25 cases of adenocarcinoma, maximum 9 cases (36%) were in grade group 5 followed by 7 cases (28%) in grade group 2, four cases (16.0%) in 3, three cases (12.0%) in 4 and 2 cases (8.0%) in grade group 1. In India, Kiruthika found maximum 48% cases were in grade group 2 and only 4% cases were in grade group 1 which was lowest among different grade groups.[13] The variation in results may be due to difference in place of sample collection. As all the samples of current study collected from DMCH which is a tertiary care hospital and most of the people were poor, they reported to the hospital at later stage of disease.

In this study neutral mucin was detected by PAS staining. In NHP 24 cases (96%) were PAS positive and 1case (4%) was PAS negative. Seven cases (28%) of adenocarcinoma were PAS positive and 18 cases (72%) were PAS negative. The difference was statistically significant ($p<0.5$). Positivity for neutral mucin was more in favor of NHP. Agarwal et al found that NHP showed positivity for PAS in 98.57% cases. In case of adenocarcinoma 56.66% cases were positive for PAS.[11] Almost similar findings were found by Bastola and Talwar in 2014. They found 87.8% cases of NHP and 44.4% cases of adenocarcinoma were PAS positive.[11]

In the present study, acid mucin was detected by Alcian blue staining. It was positive in 11 cases (44%) of adenocarcinoma out of total 25 cases. Whereas, none of the cases (0%) of NHP were positive for acid mucin. The difference was statistically significant ($p<0.5$). Agarwal et al found 46.66% cases and Khanna et al 66.67% cases of PCa were positive for acidic mucin and none of the cases were of NHP was positive for acid mucin.[11,14]

Among different grade group of adenocarcinomas 100% cases were acid mucin positive in grade group 1. Where 85.7% in grade group 2, 50% in grade group 3 and 33.3% were Alcian blue positive in grade group 4. It was absent in grade group 5. In case of adenocarcinoma our study findings correlated with the findings of Kiruthika. He observed that Alcian blue staining for acid mucin was seen in 48% cases of adenocarcinoma. Among different grade group he found 100% cases were positive in grade group 1, 50% in 2, 75% in 3, 50% in 4 and 0% in five.[13]

It was observed that all 25 (100%) cases of NHP showed positive p63 expression and all 25 cases of adenocarcinoma showed negative expression of p63 as a basal cell marker. The findings of the current study were similar with findings of Nisar et al and Kalantari et al.[15,16]

Considering the mucin histochemistry and p63 expression the current study revealed, among different grade groups of PCa, grade group 1 tumors showed 100% Alcian blue positivity, with decrease in staining with increase in grade. High grade adenocarcinomas of grade group 5 was Alcian blue negative. PAS positivity did not show any correlation with different grade group of adenocarcinomas.

All (100%) cases showed negative expression of p63 in all grade group (Table 5). By p63 we can differentiate adenocarcinoma of any grade group from benign lesions and by using acid mucin we can differentiate well differentiated carcinoma from benign lesions.

Limitations

The present study was conducted with a small sample size in a single center, so the findings might not represent the whole community

CONCLUSION

The centers where facility for IHC is available, p63 can be used as an excellent basal cell marker to differentiate NHP and prostatic adenocarcinoma. But country like Bangladesh where facility for IHC is limited and most poor people cannot take the advantage of IHC due to expense of the procedure, demonstration of acidic mucin by Alcian blue can be used to differentiate well differentiated adenocarcinoma from nodular hyperplasia. Because of the simplicity of its procedure, it can be also used in lower centers of our country.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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