Academic Journal of Cancer Research 13 (1): 01-07, 2020
ISSN 1995-8943

DOI: 10.5829/idosi.ajcr.2020.01.07

Mucin Production in Benign Hyperplasia versus Carcinoma of the Prostate

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Abstract: This study aimed at differentiating prostatic cancer from benign hyperplasia. The study was done on sixty selected prostatic specimens including 42 BPH and 18 WDPC cases. The malignant cases enclosed PIN and normal tissue foci. All studied cases were stained by PAS to detect neutral mucin and AB (at pH 2.5) to determine the acidic mucin. Results showed that all BPH cases were AB-negative and most of them were PAS- positive, however, most of WDPC were both stains - positive. AB results were potentially correlated with GS. Neutral mucin positivity's percentage was decreased from normal tissue area to WDPC, to LGPIN and to HGPIN. Also, the percentage of acidic mucin positivity was higher in WDPC than HGPIN areas while normal tissue and LGPIN zones together with BPH cases were AB-negative. Statistically significant values were detected between acidic mucin positivity of WDPC or HGPIN from one side and that of BPH or LGPIN from the other side. Conclusion: Mucin histochemical stains could differentiate benign from malignant prostatic lesions since the higher proliferative activity of malignant lesions are correlated with mucin production particularly the acidic one.

Key words: Mucin · Benign Hyperplasia · Carcinoma · Prostate

INTRODUCTION

The normal cell can be changed into a malignant one in the absence of oncogenic viral proteins [1] although the mechanism of cell regulation and molecular purposes are continuously discovered [2, 3]. Combined prostatic hyperplasia and hypertrophy include several lesions whether inflammatory or hyperplastic or neoplastic; each of which is histomorphologically characteristic [4]. The normal prostate is composed of variably sized acini lined by double cell layers; cuboidal and columnar cell layers and found in the fibromuscular rich stroma. The columnar cell layer lining the prostatic acini is secretory being dependent on androgen level [5]. A testosterone metabolite is responsible for prostatic growth and is produced within the gland through the action of the 5-alpha-reductase enzyme on the circulatory testosterone. Thus, reductase inhibitors are used as a preventive measure for prostatic hyperplastic and primary prostatic cancerous lesions and being used as a therapeutic line for benign prostatic hyperplasia (BPH) [6].

BPH as one of the most common age-related benign lesions is histologically illustrated by variably sized packed acini that may be cystically dilated and branched containing complex intraacinar papillary structures [7]. The large prostatic acini in BPH may be atypical simulating prostatic carcinoma (PC) although the cancerous prostatic acini show malignant histological features, being devoid of basal cell layer and may contain crystalloids or blue mucin in their lumen [8, 9]. It is vital to discriminate the malignant prostatic lesion that is occasionally misdiagnosed as benign until being metastasized in a near or a distant location [10].

Mucinous metaplastic acini have to be differentiated from the intraluminal mucin that is usually found in acinar adenocarcinoma of the prostate [11]. These metaplastic

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mucin-rich cells are histochemically positive for Periodic Acid Schiff (PAS) and Alcian blue (AB) [12].

Moreover, the prevalence of PC is age-related and maximally occurred with age of 75 years or older. In addition, PC discovered at the autopsy represents percentages ranging from 15 to 70% [13]. Although needle prostatic biopsy is a widespread diagnostic tool, it provides insufficient tissue for diagnostic conclusion [14].

In addition, one of the most significant factors in the prostatic cancer prognosis is to understand its histomorphological criteria [15]. However, the prostatic specific antigen (PSA) and prostatic acid phosphatase (PAP) tumor markers for PC are now less significant because they are quietly non-specific diagnostic issues [16]. In contrast, histomorphology and prostatic mucin histochemistry have fairly exhibited specific diagnostic tools for prostatic cancer as the protest of acid mucin is enormously useful in PC [17, 18]. Acidic mucin may be particularly a diagnostic marker for malignant prostatic acini as the benign ones are devoid of this mucin [13]. Mucin histochemistry is performed on the formalin-fixed and paraffin embedded tissue by using potential AB at pH 2.5 and PAS stains [4].

Intraepithelial neoplasia of the prostate (PIN); a neoplastic condition characterized by limiting the proliferated cells into the prostatic acini [19], was initially subclassified into three categories; PIN1, PIN2 and PIN3 but currently these categories have been combined into two groups; low- grade PIN group including PIN1 and high-grade PIN group involving both PIN2 and PIN3 [20]. The HGPIN lesions are considered to be the chief originator of the PC being characterized by more prominent cellular atypia with apparent nucleoli and provide several common patterns including micropapillary, cribriform, tufting and flat patterns [21]. However, the presence of peripheral basal cells in PIN acini supports its differentiation from PC that devoid of the basal cell layer [19].

Our study aimed to differentiate between BPH, PIN and well-differentiated prostatic carcinoma (WDPC) through investigation of the mucin-type secreted in these lesions by using histochemical AB (at pH 2.5) and PAS stains.

MATERIALS AND METHODS

Eligibility: A group of sixty prostatic biopsy specimens was retrospectively chosen from January 2015 to December 2017 in Pathology Department, College of Medicine, Taif University, KSA and Faculty of Medicine, Cairo University. The specimens were divided into 42 benign prostatic hyperplasia (BPH; 70%) and 18 well-differentiated prostatic carcinoma (WDPC; 30%) involving areas of prostatic intraepithelial neoplasia (PIN) and normal foci. The diagnosis of all cases was confirmed by hematoxylin and eosin (H. &E.) staining. Histochemically, all cases were studied by using Periodic Acid Schiff (PAS) and Alcian blue (pH 2.5) to determine the character of epithelial mucin production as PAS stain offered positive staining for the neutral and acidic epithelial mucin while Alcian blue (pH 2.5) stained only the acidic epithelial mucin positively. Furthermore, the combined Alcian blue-PAS was used as a confirmatory technique for the produced mucin.

Data Collection, the Ethical Aspect: This study was approved by the Ethics Committee of Taif University. There was no patient's direct contact; consequently, no informed consent had been obtained. Human subject's data had been kept in a password, protected the database and only had been linked with the identification of this research. All measures of quality control were carefully tracked.

Histochemical Technique: Prostatic tissues were fixed with neutral formalin 10%, then, dehydrated by the gradual replacement of water with alcohol by passing through increasing concentrations of ethyl alcohol (from 0 to 100%), then, the alcohol was replaced with xylene. Tissue specimens were embedded in paraffin and sectioned with a microtome at 5 µm thickness. The sections were dewaxed and rehydrated by passing them through decreasing concentrations of ethyl alcohol (from 100 to 0%). The section was dehydrated once again, put in xylene and then stained with hematoxylin and eosin (H and E) to confirm the diagnosis and to determine Gleason's score of WDPC [22]. To investigate the character of mucin, sections were stained with Periodic Acid Schiff (PAS) that only colored neutral mucin with magenta color and Alcian blue (AB; at 2.5 pH) that colored acid mucin with blue color through the reaction with its sulfate and carboxyl groups [23]. In addition, the combined Alcian blue-PAS technique was carried out to confirm the detected findings of mucin [13]. Finally, the slides were interpreted and diagnosed microscopically.

Statistical Analysis: Data was analyzed by The Chi-square test (Fisher's exact test) and p-values of less than 0.05 were considered statistically significant.

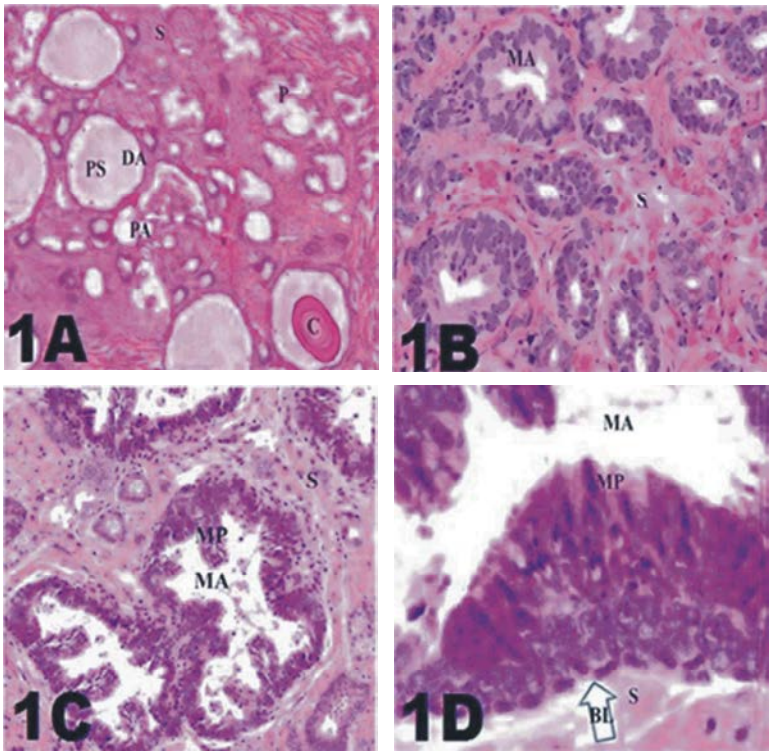

Fig. 1: Different sections of BPH,PIN and prostate carcinoma (Hx&E) **1A** is BPH showed stromal (S) and acinar (PA) hyperplasia with distended acini (DA) that contain luminal granular eosinophilic material (PS) and corpora amylaceae (C) as well as intraacinar papillary processes (P) [Hematoxylin (Hx.) and Eosin (E.) x 50]. **1B** is PC displayed malignant proliferated acini (MA) with distinctly infiltrative margin invading the stroma (S) and devoid of basal cell layer [Hx. & E.x400]. **1C & 1D** are PIN showing pseudostratified epithelial cells that lined large branched malignant acini (MA) with papillae (MP) and peripheral basal cell layer (BL) without invasion of the stroma (S) [1C; Hx. & E. x150 & 1D; Hx. & E. x1000].

Table I: Age range of patients and type of studied cases

	Studied cases (No: 60)			
Age group (ys)	No.of BPH cases	Percent (%)	No. of PC cases	Percent (%)
<40	0	0	0	0
41-60	1	2.4	0	0
61-70	35	83.3	0	0
>70	6	14.3	18	100
Total	42	100	18	100

RESULTS

All the cases were at first categorized into BPH and PC on the basis of routine microscopy. All PC cases were diagnosed as well-differentiated adenocarcinoma and possessed Gleason's scores ranged from 2-4. Most cases of BPH (35 out of 42; 83.3%) were encountered within the 7th decade of life whereas all well-differentiated prostatic carcinoma cases (WDPC; 100%) occurred after the 7th decade of life (Table I).

Histologically, BPH lesion was characterized by stromal and glandular or acinar hyperplasia, in which the glands were variably sized, crowded, cystically dilated (Fig. 1A) and infrequently contained granular eosinophilic material in their lumen. Also, the proliferated acini showed intraacinar papillary processes that were occasionally branched and joined together giving a complex pattern. In contrast, WDPC displayed either small, more or less uniform and closely packed acini or widely spaced neoplastic acini that were lined with pleomorphic cells

showing malignant histologic features and were deficient in the peripheral acinar basal cell layer (Fig. 1B). Occasionally, the neoplastic acini contained blue mucin in their lumen. Regarding PIN morphology, this lesion showed atypical pseudostratified epithelial cells that lined the large and branching acini and displayed nuclear pleomorphism, hyperchromasia and apparent nucleoli (Fig. 1C). These features simulated the malignant ones, however, basal cell layer in the PIN acini was found at the periphery (Fig. 1D).

All BPH cases were histochemically negative for AB stain while most of them (36 out of 42; 85.7%) were positively stained with PAS stain (Fig. 2A). On the other hand, most WDPC cases were stained positively with AB stain (14 out of 18; 77.8%) and PAS stain (11 out of 18; 61.1%; Fig. 2B & Table 2). Gleason score (GS) didn't correlate with the findings of PAS staining as the two PAS-negative WDPC cases possessed a GS 4 while five PAS-positive WDPC had GS 4, five PAS-positive cases had a GS2 and the remaining six PAS-positive cases had a GS 3. In contrast, all fourteen AB-positive WDPC were of a GS 4 and all four AB-negative cases had a GS 2-3. In addition, the histochemically examined foci in malignant lesions revealed decreasing order of percentages of neutral mucin production starting in the normal tissue area (66.7%), then in WDPC (61.1%; Fig. 2B), then in low-grade PIN (42.9%) and finally in high-grade PIN foci (27.3%, Fig. 2C). Conversely, acidic mucin production was seen in greater percentage in WDPC (77.8%) than in high-grade PIN (36.4%) while the normal tissue and low-grade PIN (LGPIN) zones were devoid of acidic mucin production (0%) and being AB-negative (Table 3).

In normal foci of WDPC cases, many cells of the normal acini displayed an empty foamy cytoplasm while some acinar cells showed PAS-positive fine cytoplasmic granules along their luminal border and few normal acinar lining cells exhibited larger discrete PAS-positive granules throughout the cytoplasm. All normal acinar lining cells didn't reveal AB-positive cytoplasmic material. Furthermore, the normal secretion, if found, within the acinar lumen was exposed to be morphologically globular, fibrillary or homogenous as well as PAS-positive and AB- negative (Table 3).

Acidic mucin positivity of WDPC established a statistically significant value ($P = 0.001$) when compared with that of BPH or LGPIN. Moreover, a statistically significant value ($P = 0.001$) was found in acidic mucin positivity of HGPIN when matched with that of either BPH or LGPIN. A statistically insignificant value ($P = 0.05$) was recognized in acidic mucin positivity of WDPC concerning that of HGPIN. Also, an insignificant statistical value ($P = 0.05$) was detected in comparing the neutral mucin positivity of any two of the studied prostatic lesions.

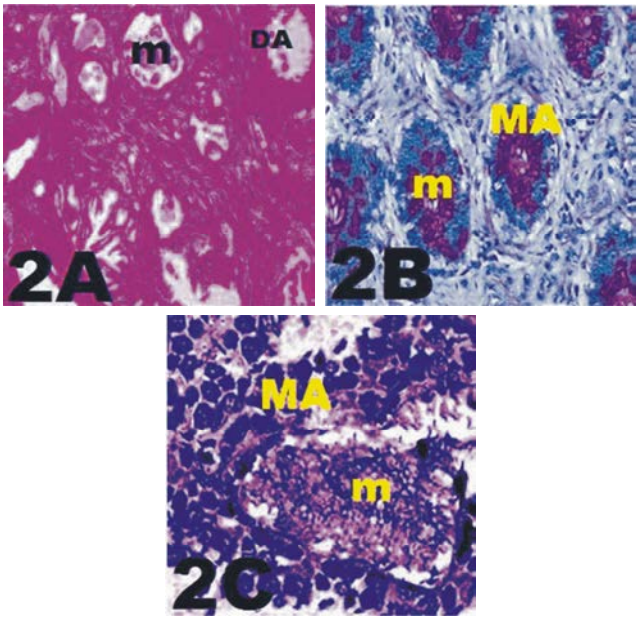

Fig. 2: Sections in prostate BPH & PC (PAS &AB satins) **2A** is BPH showed a positively stained neutral mucin (m) within their distended acini (DA) [Periodic Acid Schiff (PAS) x 50] while the malignant acini (MA) in the PC (**2B**) and PIN (**2C**) showed positively stained acidic and neutral mucin (m) [Combined Alcian Blue (AB)/ PAS stain; 2B x200 and 2C x1000].

Table 2: Mucin staining in studied prostatic lesions

Lesion	Total No; of cases	Neutral Mucin PAS-positive		Acidic Mucin AB-positive		
		No;	%	No;	%	*P* value
Benign prostatic hyperplasia (BPH)	42	36	85.7	0	0	0.001*
Well-differentiated prostatic carcinoma (WDPC)	18	11	61.1	14	77.8	

* *P* value between acidic mucin positivity of WDPC and that of BPH was 0.001which is significant.

Table 3: Percentage of mucin positive staining in various tissue foci in WDPC cases

Foci of tissue	Total No; of cases (n=18)			
	Neutral mucin PAS-positive		Acidic mucin AB-positive	
	No;	%	No;	%
Normal tissue (NT)	12/18	66.7	0	0
Well-differentiated prostatic carcinoma (WDPC)	11/18	61.1	14/18	77.8
Low-grade intraepithelial neoplasia (LGPIN)	3/7	42.9	0	0
High-grade intraepithelial neoplasia (HGPIN)	3/11	27.3	4/11	36.4

**P* value between acidic mucin positivity in HGPIN foci and that in LGPIN foci was 0.001; **P* value between acidic mucin positivity in WDPC foci and that in LGPIN foci was 0.001which is significant.

DISCUSSION

Discrimination between BPH and prostatic cancer is greatly essential since both lesions may have identical clinical features and mechanisms of pathogenesis. In addition, these lesions depend broadly in their management on pathological and radiological detection of Gleason's score, the serum level of PSA and other analytic measures that were unreliable and insufficient [24]. Therefore, we detected the potential role of certain histochemical and morphological stains in patients with prostatomegaly. Demographically, our study revealed that most BPH cases were encountered in the age group ranged from 61-70 years while all cases of the prostatic carcinoma occurred above the age of 70 years. These results simulate those reported by Anchit *et al*. [4] and Durgaprasad *et al*. [13].

Alcian blue at pH 2.5 was used as a specific stain for the synthesized epithelial cell non-sulfated mucin containing sialic acid as mentioned by Anchit *et al*. [4] Although we didn't detect acidic mucin in any case of BPH, a finding identical to that was stated by Durgaprasad *et al*. [13] and Pinder and McMahon [25] who detected this mucin in 5% of their studied BPH cases, however, other investigators detected this type of mucin in a significant percentage of their studied BPH cases [17]. On the other hand, the study displayed a potential correlation of the prostatic carcinoma cases and their Gleason's score as the acidic mucin noticed in WDPC had possessed higher Gleason's score than WDPC cases which were negative for acidic mucin staining. Moreover, in AB-positive WDPC, acidic mucin was found in 72.2% of the carcinomatous areas and in 38.9% of the high-grade intraepithelial neoplastic foci. These findings are similar to those stated by Durgaprasad *et al*. [13] and contrasted from the results of Pinder and McMahon [25] who reported that acidic mucin was elucidated in only 38% of the prostatic carcinoma. We didn't identify acidic mucin in either normal areas or in low-grade intraepithelial neoplastic foci. This finding is identical to that explained by Anchit *et al*. [4] and Mathur *et al*. [17] and who detected that the acidic mucin positivity had an essential role in differentiating between atypical adenomatous prostatic hyperplasia, prostatic intraepithelial neoplasia and prostatic carcinoma since these lesions could show histological resemblances. In addition, they discovered greater secretion of acidic mucin in carcinomatous than in benign disorders, premalignant lesions or normal tissue of the prostate. We, as well, observed a statistical significance (p=0.001) in acidic mucin staining positivity between BPH and WDPC and between HGPIN and LGPIN tissues indicated an increase in the acidic mucin synthesizing capacity with the higher grade of cellular anaplasia.

Alternatively, we found that PAS stain didn't distinguish between benign, premalignant or malignant prostatic disorders and it didn't correlate with Gleason score of the malignant prostatic lesions. Furthermore, decreasing order of PAS positivity percentages for neutral mucin production was insignificantly clarified in the normal, carcinomatous, LGPIN and HGPIN tissue areas. Our records are coincident with those reported by Anchit

et al. [4] and Mathur *et al*. [17] who itemized that both AB and PAS staining, not only one of them, can be used for a more truthful diagnosis in those microscopically overlapped ordinarily stained benign, premalignant and malignant prostatic cases. In addition, they mentioned that PAS stain has been yet valuable in approving the prostatic origin of both primary malignant poorly differentiated and metastatic tumors.

CONCLUSIONS

Specific histochemical Alcian blue (pH 2.5) and Periodic Acid Schiff stains are useful in the determination of the character of secreted prostatic epithelial mucins. Histochemical Alcian blue (pH 2.5) stain can be used as easy low-cost method to histologically discern the suspicious benign from well-differentiated malignant prostatic lesions.

ACKNOWLEDGMENTS

We would like to acknowledge Department of Pathology, College of Medicine, Taif University, KSA and Faculty of Medicine Cairo University for their help and support this work.

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