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Original Research Article

Utility of Mucin Histochemistry in Prostatic Neoplasms with Review of Historical Background

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Abstract

Introduction: The prostatic cancer is the second most common cause of cancer death in men.

Aim: To elaborate the study of mucin histochemistry in prostatic lesions and establish theutility of Mucin Stains in distinguishing benign lesions from in situ and malignant lesions.

Materials and methods: In the retrospective and prospective study. Cases were admitted and biopsied who underwent prostatic biopsies and surgeries due to various prostatic pathologies. Biopsy specimen paraffin blocks of the tissue were obtained and fresh sections were made with a thickness of six microns on a rotary microtome. All the sections were stained with Hematoxylin and Eosin.

Results: A five year combined retrospective and prospective study from 1 st January 2012 to 31 st October 2016, revealed a total of 347 prostate biopsies. The total number of biopsy specimens received during the above five year period were 13338, and the prostate biopsies accounted for an overall proportion of 2.6 %. 89% of cases in study are malignant and 21% are benign.

Conclusion: Mucin histochemistry evaluation is a simple, reliable and economical technique to confirm the presence of malignancy in suspicious lesions.

Keywords: Mucin Histochemistry; Prostate Lesions; Alcian Blue-PAS Stain.

Introduction

Prostate gland specimens account for a significant percentage of diagnostically challenging cases in surgical pathology practice. The understanding of prostate pathology has progressed.

The spectrum of prostate lesions range from inflammatory, bengin, pre malignant and malignanant lesions. Among all, benign prostatic enlargements are the commonest and constitutes an important clinical entity. The natural history of Benign Prostatic Hyperplsia is complex and multifactorial followed by the carcinomas Stamey 1 considered prostatitis as “waste basket of clinical ignorance”.

Much of the time the histological diagnosis is made without difficulty on H&E stained sections. However in two instances like presence of Considerable crush artifacts of an otherwise typical acinar type of adenocarcinoma and secondly when the tumor is so well differentiated as to be confused with atypical epithelial hyperplasia; some other simple, economical and reliable diagnostic methodology should be available to the practicing pathologists. That aid is Mucin histochemistry.

It is an ancient method that can be performed routinely using availble techniques within a laboratory with basic facilities.Much emphasis and work was done on "Mucin histochemistry" in various tissues by different authors. Stacey & Barker (1962) reported that these Muco substances are of three main types-

1. Mucoprotein.
2. Mucopolysacharides.
3. Mucolipids.

The present study is an attempt to study the mucin staining pattern in Benign, Pre – malignant & Malignanat lesions of prostate and to highlight its diagnostic importance, using simple techniques available in the histopathological laboratories.

Aim of the Study

To elaborate the study of mucin histochemistry in prostatic lesions and establish the utility of Mucin Stains in distinguishing benign lesions from insitu and malignant lesions.

Materials and Methods

In the retrospective and prospective study. Cases were admitted and biopsied at Kamineni Hospitals L.B Nagar, Hyderabad between January 2012 to 31 st October 2017 who underwent prostatic biopsies and surgeries due to various prostatic pathologies Teaching Hospital. Biopsy specimen paraffin blocks of the tissue were obtained and fresh sections were made with a thickness of six microns on a rotary microtome.

All the sections were stained with Hematoxylin and Eosin. In addition, sections were also stained with special stains to identify the various mucins,such as acid and neutral mucins. Alcian blue at Ph 2.5 and Muller's colloidal Iron stain for the detection of acid mucins, and neutral mucins were detected by PAS. Relevant clinical data were collected. Data was coded and entered into Microsoft excel and analysed.

Result

In 5 years total 347 biopsies were obtained and included in study. A five year combined retrospective and prospective study from 1 st January 2012 to 31 st October 2016, revealed a total of 347 prostate biopsies.

The total number of biopsy specimens received during the above five year period were 13338, and the prostate biopsies accounted for an overall proportion of 2.6%. 89% of cases in study are malignant and 21% are benign.

Table 1: A five year study of prostate lesions in 347 prostate biopsies

Period	Prostate Biopsies	Total Biopsies
1-1-2012 to 31-12-2012	51	3153
1-1-2013 to 31-12-2013	126	2110
1-1-2014 to 31-12-12014	59	2490
1-1-2015 to 30-11-2015	68	3113
1-1-2016 to 31-10-2016	43	2472
Total	347	13338

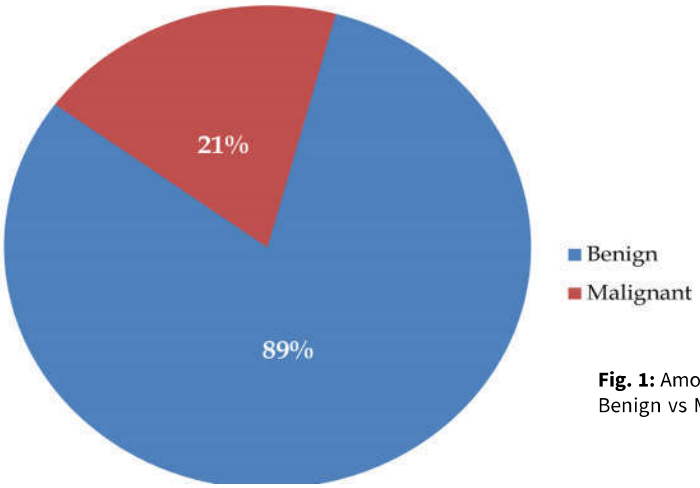

Fig. 1: Amount of neutral & acid mucins- Benign vs Malignant

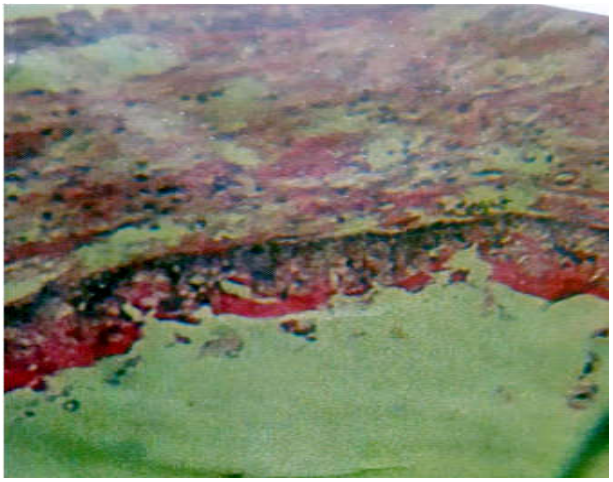

Fig. 2: Photomicrograph with Demonstration of neutral mucins in benign prostate hyperplasia(BPH)-40x Alcian Blue –PAS method

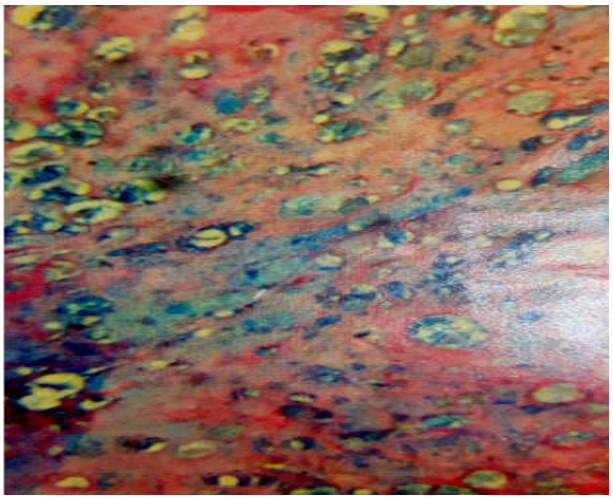

Fig. 3: Photomicrograph with Demonstration of intraluminal Acid mucins-40x Muller's "Colloidal Iron stain".

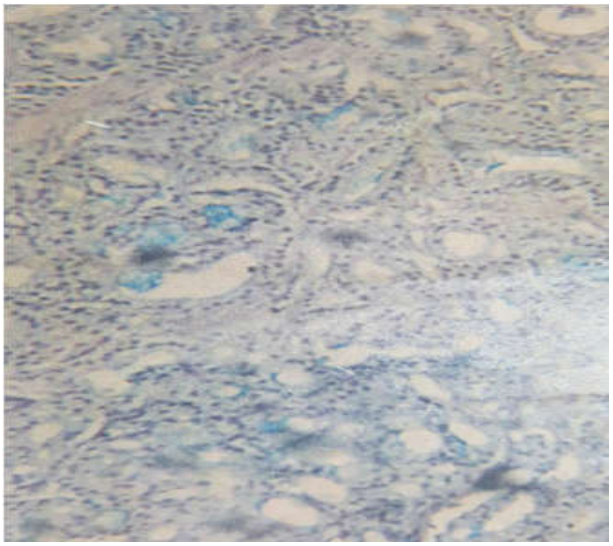

Fig. 4: Photomicrograph with Demonstration of intraluminal Acid mucins in prostate carcinoma& an adjacent area of atypical adenomatous hyperplasia(AAH)&PIN -III-40x AB-PAS Technique.

Discussion

"Mucin" has been a familiar word among tissue pathologists for many years but, little was known about the chemistry & histochemistry of the material until quite recently.

The original term "Mucin" was mentioned in a book by an American worker named Carpenter as long ago as 1846 [6]. Much later the term changed to "Mucins" as the complex nature of these substances began to emerge. Other terms followed over the years and these included "Mucopolysaccharides" , Mucosubstances [7].

Mayer (1930) classified the mucosubstances of the body and characterized the chemical properties of various mucins [6].

More recently, the term "glycoconjugates" has been suggested as a general name to replace those already mentioned [6]. Briefly, their suggested redefinition is that glycoconjugates be subdivided into "Proteoglycans" & "Glycoprotein". Put simply, muco substances are hexosamine – containing poly saccharide covalently bound to varying amounts of protein. The different Mucins may be present as a single type within a given tissue unit or more usually as a mixture of different types. The synthesis of mucin is initiated in the rough endoplamic reticulum and completed in the Golgi – apparatus. Mucins can be demonstrated at electron microscopic level and techniques incorporating an electron dense metal, either free or as part of a dye molecule are utilized, these include Alcian blue (at different Ph levels), Ruthenium Red & dialysed Iron.

Blix, Gots Chalk and Werner [7] identified the specific carbohydrate linked to the proteins of certain kinds of epithelial mucin and specified the mode linkage. Diagnostic pathologists have long used various stains for – "Mucin" in an effort to identify carcinomas more precisely and to ascertain the tissue of origin. In the past, the help obtained from mucin stains has largely been disappointing because of the many different kinds of Muco substances found in tumors and the varying spectra of sensitivity of the different stains.

Much progress has been made in the past 30 years in elucidating the chemical composition of Muco substances produced by various mammalian epithelial cells. Concurrently histochemical methods have been developed that are capable of discriminating among several classes of mucin and in certain instances of identifying some of their chemical components with specificity.

Modern histochemical study of mucosubstances began with the periodic acid Schiff stain of Hotchkiss and Mc Manus [8]. Small amounts of mucin confined within the lumina can be demonstrated in a great number of prostatic neoplasms.

Elbadaur et. al. [10] reported that neutral mucins may be secreted in small amounts by prostatic epithelial cells whether benign or malignant and they are present in cytoplasmic granules. They are variably eosinophilic and are stained by the PAS technique. Franks (1954)[11], Poster & Levine (1964)[12] & Levine & foster(1963) [13]– demonstrated acid mucins in the acini of some well differentiated prostatic cancers and also within atrophic hyperplasia (Franks 1954) [11].

Hukill et al stated that acidic mucins were rare and scant in benign prostate gland lumina, their abundant secretion is specific for mucinous areas of prostatic cancer. They are usually faintly basophilic with H & E stain; but they are specifically identified by Alcian blue technique which does not stain the neutral mucin.

Hukill & Vidone (1967) [14] reported that 78% of carcinoma of prostate produced a combination of a neutral mucin (very rare) and an acid mucin (sulphated and non – sulphated forms). Peterson (1909) [15]– postulated that, presence of acid mucin represents a disturbance of the normal secretory cycle. Normal prostate synthesizes a neutral mucin, because sperms survive and are mobile in that type of PH medium. The pattern and intensity of staining for non sulphated acidic mucin appeared to be similar to that for total acid mucin in Atypical Adenomatous Hyperplasia & Cancer, there by indicating a close relationship in mucin expression between Atypical Adenomatous Hyperplasia & well differentiated adenocarcinoma. This is useful in separating some cases of Atypical Adenomatous Hyperplasia & adenocarcinoma from benign prostatic epithelium (Goldstein & Bostwick D.G) [16].

Luna More S et. al. [17] postulated that the incidence of acid mucins was higher in prostatic carcinoma as compared to Atypical Adenomatous Hyperplasia & directly proportional to the tumorgrade.It is suggested that Atypical Adenomatous Hyperplasia positive for acid mucins should be classified in the high grade Atypical Adenomatous Hyperplasia group.

Morphological and histochemical analysis by Mc Neal [18] revealed mucin secreting areas in 63 % of prostatic adenocarcinomas. He also observed Gleason grade 3 & 4 patterns in adenocarcinoma with focal mucinus component.

Taylor [19] reported that the presence of acid mucin is of diagnostic significance.

Ro et. al. [20] agrees that, stain for acid mucins are useful in confirming the diagnosis of prostatic cancer especially in equivocal cases in which severe crush artifacts are present or in a small biopsy material. If the mucinous area occupies < 20% of the tissue volume of any cancer it should be designated as prostatic adenocarcinoma with a focal mucinous component.

Mc Neal et. al. (1995) [18] reported the other lesions that also secrete acidic mucins: like mucinous metaplasia, PIN, sclerosing adenosis and basal cell hyperplasia.Gold stein et al (1995) [16] found 63% of glands are positive for mucins in AAH. Sentinelli [21] found similarities in the mucin staining pattern between PIN III and prostate adenocarcinoma.

Taylor reported that the findings of mucins in a distant lymph node does not aid in the differential diagnosis of a metastatic adenocarcinoma because the pattern of mucus production by prostatic tumor is so variable and other common tumors produce similar mucoprotein; but when a prostatic lesion is of questionable malignancy – identification of mucoprotein are of definite help.

Several types of mucoprotein exist in different histological patterns of prostatic carcinomas or lesions and it serves no purpose here to differentiate them by specific staining of sulphated or non –sulphated sialic acid residues as was done by Franks et. al. [12], Hukill & Vidone[14] , K.B. Logani et. al. [22], H.L. Arora [23].

The role of histochemistry in poorly differentiated or anaplastic carcinoma of the prostate is very limited, because in advanced carcinoma the secretion disappears.

Acid mucins are seen in better differentiated tumors, which tend to grow more slowly and to present at a more favourable stage of the disease. Therefore identification of the types of mucins have a definite prognostic and diagnostic value.

In the present study of 347 biopses, the differentiation was mainly between the neutral mucins and the acid mucins. In cases of benign hyperplasia, the amount of neutral mucins was 89 % and the amount of acid mucins was 21 % (Figure 1) indicating the abundance of neutral mucins and less abundant acid mucins.

Conclusion

Mucin histochemistry evaluation is a simple ,reliable and economical technique to confirm the presence of malignancy in suspicious lesions. The presence of acidic mucin would be useful in confirming the diagnosis of prostate cancer in equivocal cases in which severe crush artifacts when present (or) in a small biopsy specimen.

Identification of the types of mucins with the (combined Alcian blue at ph 2.5 PAS & High iron diamine method) and their expression in various neoplastic lesions of the prostatic forms an important adjuvant diagnostic test , to distinguish benign prostatic glands from the glands of well differentiated adenocarcinoma of the prostatic, in this era of modern immunohistochemistry and molecular level of cancer detection.

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