

Histochemical Characteristics of Mucosubstances in Diseased Breast

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Abstract

Background: The apical epithelial surfaces of mammalian respiratory, gastrointestinal, and reproductive tracts are coated by mucus, a mixture of water, ions, glycoproteins, proteins, and lipids. Mucins are mainly of two types- Neutral and Acidic. **Objectives:** To know the mucin distribution of the breast in malignant lesions. **Methodology:** This is a retrospective, observational, analytical, case control study aimed to evaluate mucin histochemical pattern in malignant breast. Twenty five histologically proven malignant human breast tissue were taken. Tissue sections were stained by Mayer's Haematoxylin and Eosin, PAS, PAS-diastase, Phenylhydrazine-PAS, Alcian blue 2.5, Alcian Blue 1, combined alcian blue-PAS, Aldehyde Fuchsin and combined aldehyde fuchsin-alcian blue techniques. **Results:** Results were tabulated according to color intensity into different grades ranging from + to +++. Regarding mucin histochemistry of malignant breast - "sialomucins" were seen predominant than neutral and sulphomucins. **Conclusion:** Mucin histochemical patterns have valuable, cost-effective, and important role where a slight change in the mucin pattern may help in the early diagnosis of the disease process. Mucin histochemistry may provide a valuable and cost-effective tool for the diagnostic histopathology and for the researchers in histology.

Keywords: Mucosubstances; Special Stains; Breast Mucins; Carcinoma.

Introduction

Mucus is a complex viscous adherent secretion synthesized by specialized goblet cells in the columnar epithelium that lines all of the organs that are exposed to the external environment. This includes the respiratory tract, the gastrointestinal tract, the reproductive tract and the oculo-rhinolaryngeal tracts [1,2]. Mucus is composed primarily of water (95%), but also contains salts, lipids such as fatty acids, phospholipids and cholesterol, 1 proteins which serve a defensive purpose such as lysozyme, immunoglobulins, defensins, growth factors and trefoil factors. However, the main component that is responsible for its viscous and elastic gel-like properties is the glycoprotein mucin [1]. Mucins are high molecular weight

glycoproteins that are found dispersed throughout the epithelia of the gastrointestinal, respiratory and reproductive tract [3]. The term mucosubstances is used, as recommended by Spicer, Leppi and Stoward (1965), to denote all tissue components other than glycogen, rich in carbohydrates, which are present in connective tissue or as secretion of certain epithelial structures [4]. Connective tissue mucosubstances are called "mucopolysaccharides", while those secreted by epithelia are referred as "mucins" [5]. Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues in most metazoans [6]. 'Mucins' key characteristic is their ability to form gels; therefore they are a key component in most gel-like secretions, serving functions from lubrication to cell signalling to forming chemical barriers. They often take an inhibitory role [6]. The function of mucins varies in part upon the tissue location of the mucin producing cell as well as the mucin type. In most cases, the secreted mucins provide lubrication and protection for the secreting cells in the immediate area. The function or role of the membrane bound mucins is not well understood. These mucins are likely involved in the regulation of cellular functions such as cell proliferation and cell adhesion [7]. Mucins perform a wide variety of functions like lubrication, protection against acids etc. The mucosubstances also contain immunoglobulins primarily of IgA type, lactoferrin

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which chelate the iron necessary for growth of some bacteria and lysosomes which destroy some of the bacteria. Hence they act as antibacterial and antiviral agents and have protective mechanism [8]. Mucins are classified into two main categories namely, Neutral mucins and acidic mucins. Neutral mucins are slightly alkaline in nature and mainly help for reducing the pH and toxicity of substances. They are first to appear during development in intrauterine life by fourth to fifth month. Acidic mucins are subclassified into weakly acidic and strongly acidic [9,10,11]. Weakly acidic mucins contain terminal carboxyl groups and are called as carboxylated mucins or sialomucins. They contain chelating agents and have antibacterial and antiviral property. Strongly acidic mucins contain sulphate groups and are called as sulphomucins. They are thick, viscous and help for formation of protective coat for lubrication [10,11]. Histochemistry is defined as any technique in which a chemical reaction is involved in coloring tissue, be it staining with dyes or not. Thus more properly we have 'non-dye' histochemical technique and 'dye-involved' histochemical technique. The designation of a stain as special may be arbitrary, but generally any stain other than H and E is regarded as special stain. They are used in an attempt to identify cell and tissue components by virtue of their specific chemical reactions [11].

In malignancy, the malignant cells change their behavioural pattern and secrete different types of mucin than normal. During carcinomatous changes, cells revert back to their embryonic stage. Secretory changes occur even before the nuclear changes are visible and hence study of mucins may help to identify cancerous conditions at an early stage [12]. Thus early diagnosis even before carcinoma in situ will be of great clinical value in reducing the morbidity and mortality in the patients. Mucosubstances of human mammary gland but few have studied about the mucin histochemical characteristics in Intra Ductal Carcinoma using the stains used in present study. So the present study has been undertaken and correlated with previous workers [13-17].

Material and Methods

The present study was conducted in the Department of Anatomy, Krishna Institute of Medical Sciences, University, Karad from May 2014 to June 2017. The type of study was observational, analytical and case control study. Sample size was 30 blocks of each normal and carcinoma colon. The study was

performed on 25 specimens of histologically proven infiltrating duct carcinoma of breast collected from postmortems and surgically removed specimen from Krishna Hospital Siddhivinayak Cancer Hospital, Miraj. The tissues were fixed in 10% formal saline with 2% calcium acetate and a pinch of phosphotungstic acid to help for preservation of mucins.

The tissues were embedded in paraffin and blocks were prepared by histopathological technique and cut at 5-6 microns. Sections were stained with Hematoxylin and Eosin, and the following histochemical methods were performed on paraffin-embedded sections for the characterization of different mucosubstances as PAS, PAS diastase, PAS- Phenyl hydrazine, Alcian blue (AB) - pH 1 and 2.5, Aldehyde fuchsin (AF), combined AB-PAS and combined AF-AB.

1. P.A.S. – Periodic acid Schiff reagent stains all carbohydrates including mucosubstances. Therefore mucosubstances are P.A.S. positive.
2. P.A.S. Diastase – Diastase dissolves glycogen like carbohydrates, but mucin remains unaffected.

This stain is used for confirmation of mucosubstances.

3. P.A.S. Phenyl hydrazine – Phenyl hydrazine dissolves neutral mucosubstances only and hence to prove their presence.
4. Alcian blue – This stain can be used at various pH levels.
 - a. AB pH 1 – This stain is highly acidic and stains sulphomucins only.
 - b. AB pH 2.5 – This stain is weakly acidic and stains both carboxylated and sulphomucins.
5. Aldehyde Fuchsin – This stain only stains sulphomucins and confirms their presence.
6. Combined AB-PAS – This staining procedure will stain all different types of mucin. Neutral – Magenta, Carboxylated – Blue, Sulphated – Purple.
7. Combined AF-AB – This staining procedure helps for differentiation and confirmation of carboxylated and sulphated mucins.

Carboxylated – Blue, Sulphated – Purple

All the results obtained were tabulated according to color intensity into different grades ranging from + to +++++. [18,19,20].

1. +++++: Very strong positive reaction.
2. ++++: Strong positive reaction.

3. ++ : Moderate reaction.
4. + : Weak reaction.
5. No staining: Negative reaction

Observation and Results

In the present study, H and E and special stains were used for mucinhistochemistry of 25 specimens of infiltrating duct carcinoma of breast.

Haematoxylin and Eosin was used to identify and confirm the diagnosis of carcinoma.

1. Sections studied show a tumor composed of cells arranged in diffuse sheets, well-defined nests, cords and as individual cells.

At places glandular differentiation is seen to be well developed while in other areas it is barely detectable or altogether absent.

The tumor cells are large and exhibit pleomorphism. Their nuclei and nucleoli are prominent. Numerous mitotic figures are seen.

Areas of necrosis are noted. Abundant stroma is seen and appears to be densely fibrotic to cellular.

A mononuclear inflammatory infiltrate of variable density is seen in the interphase between tumor and stroma (Figure 1).

2. PAS stain was used to assess presence of neutral mucosubstances. PAS stain gave mild reaction as focal magenta staining, suggestive of presence of few neutral mucosubstances (Figure 2).
3. PAS-diastase was used for confirmation of mucosubstances as diastase dissolves glycogen like carbohydrates but mucins remained unaffected. This gave mild to moderate reaction as magenta color confirming the presence of few PAS positive mucins (Figure 3)
4. PAS-Phenyl hydrazine was used to prove the presence of neutral mucosubstances as Phenyl hydrazine dissolves neutral mucosubstances only. PAS - Phenyl hydrazine gave mild to moderate reaction as magenta color confirms few neutral mucosubstances (Figure 4).
5. AB 2.5 pH gave mild reaction as blue colour . This is suggestive of presence of trace amount of acidic mucosubstances (Figure 5).
6. When stained with AB 1, To differentiate between the two, carboxylated (weakly acidic) and sulphated (strongly acidic) Alcian blue pH1 was carried out. AB-pH1 showed no reaction suggestive of no sulphatedmucins (Figure 6).

7. Aldehyde fuchsin stains only sulphomucins and confirms their presence . AF gave weak reactions as faint purple color suggestive of presence of few sulphatedmucins (Figure 7).
8. AB-PAS stain gave weak reaction as blue suggestive of trace amount of acidic mucosubstances. (Figure 8).
9. AF-AB technique helps to differentiate and confirm carboxylated and sulfated mucins. It gave strong reaction as blue and weak reaction as purple colour suggestive of predominance of carboxylated mucins and traces of sulphatedmucins (Figure 9).

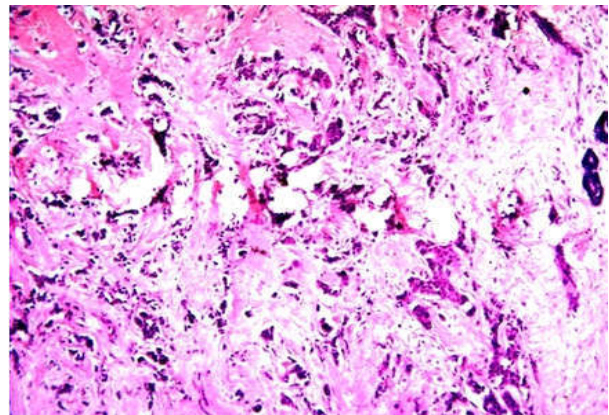


Fig. 1: Histology of Invasive Ductal Malignancy of Breast Photomicrograph 1 (H&E,10X)

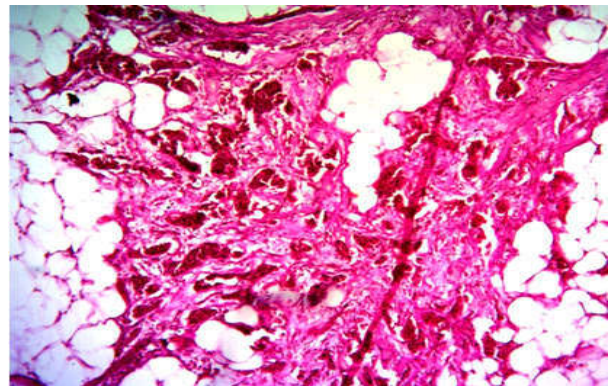


Fig. 2: PAS Photomicrograph 2 (H&E,10X)

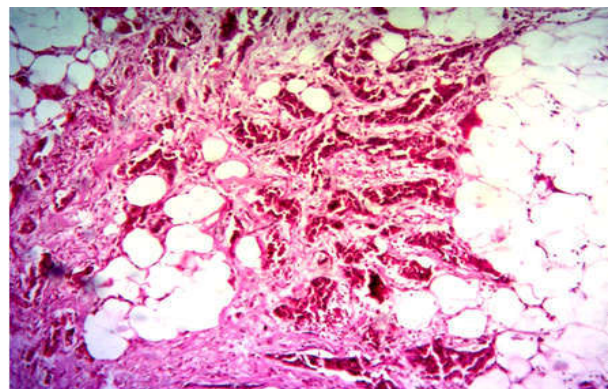


Fig. 3: PAS D Photomicrograph 3 (H&E,10X)

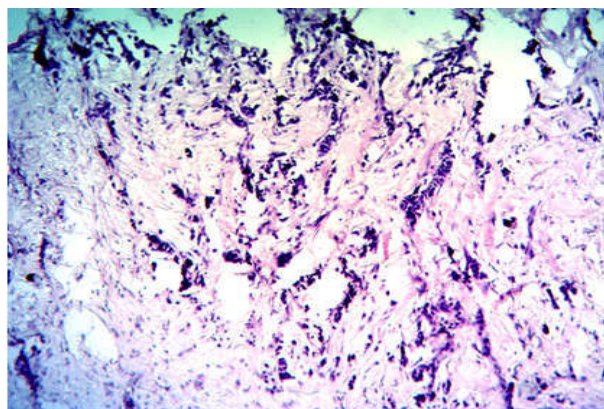


Fig. 4: PAS PH Photomicrograph 4 (H&E,10X)

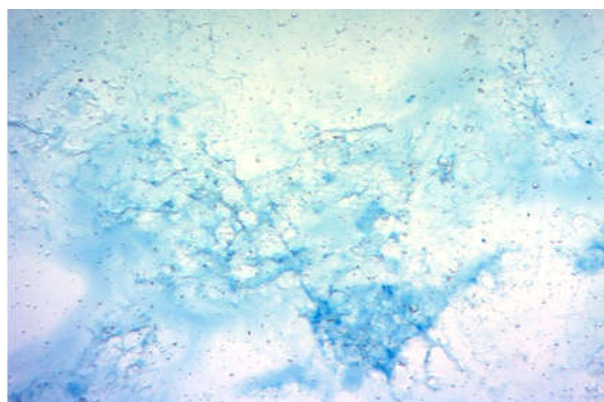


Fig. 5: AB 2.5 Photomicrograph 5 (H&E,10X)

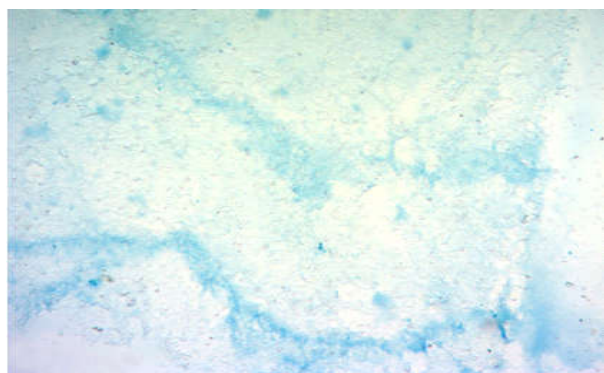


Fig. 6: AB 1 Photomicrograph 6 (H&E,10X)

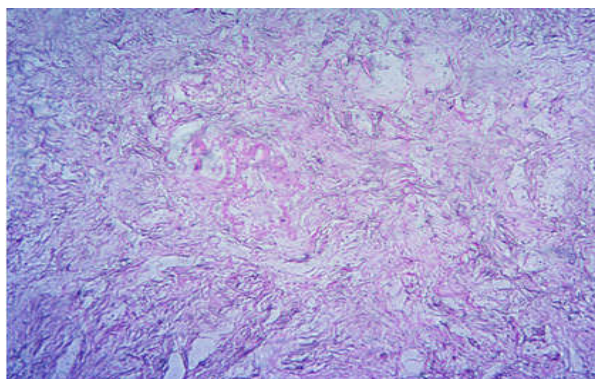


Fig. 7: AF Photomicrograph 7 (H&E,10X)

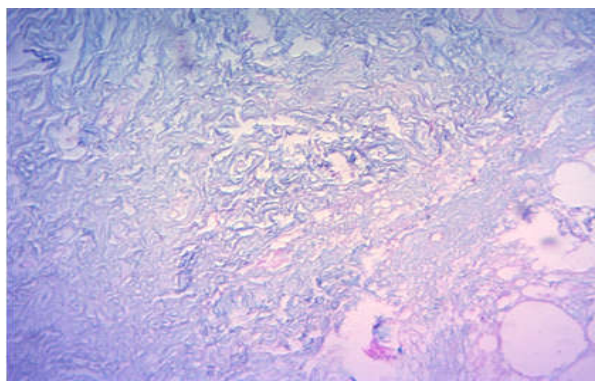


Fig. 8: AB PAS Photomicrograph 8 (H&E,10X)

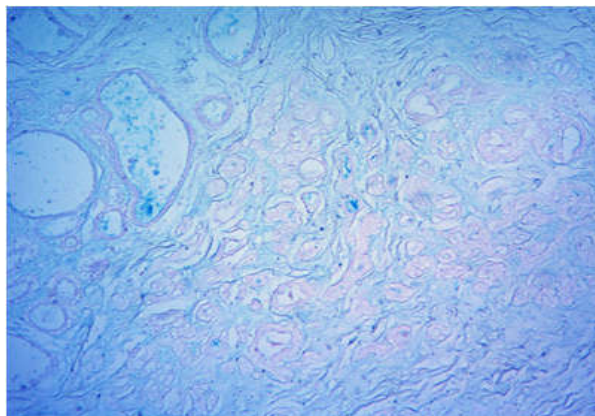


Fig. 9: AF AB Photomicrograph 9 (H&E,10X)

Table 1: Showing results of Mammary gland staining

No.	Stain	Intensity	Inference
1	H&E	-	Invasive Ductal carcinoma identified and confirmed.
2	PAS	+++	Presence of PAS positive substances i.e. carbohydrates and neutral mucins.
3	PAS-D	++	Presence of glycogen.
4	PAS-PH	+ / -	Presence of large amount of neutral mucins.
5	AB 2.5	+++	Presence of acidic mucins.
6	AB 1	+	Presence of sulfomucins in trace amount.
7	AF	+	Confirms presence of sulfomucins
8	AB-PAS	Magenta +++ Blue ++	Presence of neutral and acidic mucins.
9	AF-AB	Blue ++ Purple +	Confirms presence of sialomucins with trace amounts of sulfomucins.

Regarding mucinhistochemistry of infiltrating Duct Carcinoma of Breast, "mixture of mucosubstances" were observed, "carboxylated acidic mucins" were seen predominantly than neutral and sulphated acidic mucins.

Discussion

Mucin is a high molecular weight glycoprotein that is synthesized, stored and secreted by connective tissue and epithelial mucosal cells, especially the goblet cells [23]. In normal tissues, mucins seem to exhibit tissue- and cell-specific histochemistry patterns of expression.

The patterns of distribution exhibited might be quite complex, with several different mucins often expressed in the same organ and at times the same cell [24]. However, under pathologic conditions this distinct expression patterns are modified. Numerous types of mucin occur depending on the site of production. Examples of connective tissue mucins are chondroitin sulphate, heparin sulphate, keratansulphate, and hyaluronic acid. Epithelial mucins may be acid or neutral. Neutral mucins are hexosamine units without free acidic groups. Acid mucins consist of hexosamine units which may be associated with glucuronic or sialic acid, the reactive group being a carboxyl. In sulphated mucins this group is blocked by a sulphate group which becomes the active group. Strongly sulphated mucins are of connective tissue type; the weakly sulphated groups are of epithelial type. The non-sulphated mucins are sialic acid and hyaluronic acid (carboxylated D-glucuronic acid). These can be enzymatically digested, though enzyme-resistant forms do occur. The presence of carboxyl groups or sulphate groups was determined by the various staining techniques described and confirmed using enzyme digestion methods. Until relatively recently the type of mucin in mucous carcinoma of the breast has remained in doubt. It is of interest that Lange (1896) [25], who stained his sections with toluidine blue, concluded that the mucin was of connective tissue origin. However, Gaabe (1908) [26] using the same stain concluded that the mucin was of epithelial origin, a theory propounded by Virchow (quoted by Lee, Hauser, and Pack, 1934) [27]. Ewing (1922) [28] favoured a dual origin from both connective tissue and epithelium but also in some cases from fat. The first histochemical study performed in order to ascertain the type of mucin was by Grishman (1952) [29] who demonstrated its epithelial origin. Since then many histochemical studies of mucous carcinoma of

the breast have appeared in the North American literature. Norris and Taylor (1965) [30] found the mucin to be a poorly sulphated mucopolysaccharide, though Spicer, Neubecker, Warren, and Henson (1962) [31] had shown this to be enzymatically digestible sialic acid. The present study has confirmed the presence of sialomucin, but abundant neutral mucin has also been demonstrated. The acid mucin was of carboxylated type which is the most commonly encountered type in IDC breast. This study is in accordance with the above mentioned worker. Occasionally a mammary carcinoma may show marked mucin production within the stroma. However, apart from the different histochemistry of stromal mucin, the histological appearances are distinct from that of a mucous carcinoma.

Conclusion

The Invasive Ductal Carcinoma showed the presence of neutral mucins and acidic mucins. In acidic mucin sialomucins were present and sulfomucins were recorded in trace amounts. Any change in the mucin pattern may be helpful in the early diagnosis of any disease process.

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