Original Article

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Histochemical Characteristics of Mucosubstances in Normal Human Mammary Gland

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Abstract

Introduction: Mucins implicated in cancers of various organs. The apical epithelial surfaces of mammalian respiratory, gastrointestinal, and reproductive tracts are coated by mucus, a mixture of water, ions, glycoproteins, proteins, and lipids. The purpose of this study was to confirm the presence of mucin production using Haematoxylin and Eosin (H&E) stain as the gold standard and to describe the types of mucins using various types of histochemical techniques. *Method:* This is a retrospective, observational, analytical, case control study aimed to evaluate mucins histo-chemical pattern in normal human breast. Twenty five histologically proven normal 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 Fuschin and combined aldehyde fuchsin-alcian blue techniques. *Result:* The human mammary gland showed the presence of neutral mucins and acidic mucins. In acidic mucins sialomucins were present and sulfomucins were recorded in trace amount. This concludes that 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.

Keywords: Mammary Gland; Mucosubstances; Special Stains; Breast Mucins.

Introduction

The mammae exit in both sexes.In male they are rudimentary throughout life; in the female they are underdeveloped before puberty but undergo considerable growth and elaboration at and after puberty.In the lateral plane its base extends vertically from the second to the sixth rib, andat the level of the fourth costal cartilage it extends transversely from the side of the sternum to near the midaxillary line [1].

Histologically, mammary glands are modified tubuloalveolar apocrine sweat glands. The tubuloalveolar mammar glands, derived from modified sweat glands in the epidermis, lie in the subcutaneous tissue. The inactive adult mammary gland is composed of fifteen to twenty irregular lobes separated by fibrous bands of connective tissue [2]. These alveoli secrete Mucosubstances which performs a wide variety of functions like lubrication, protection against acids, maintenance of hydration etc. They also contain immunoglobulin's mainly IgA type, lactoferrins which chelate the iron necessary for growth of some bacteria and lysozymes which destroy some of bacteria. The mucins play an important role defense against bacteria. Sulphomucins are acting as antiulcerogenic as they coat and protect mucosal surface while neutral mucins help for secretion of enzymes [3]. Many workers like Soo Youn Bae [4], D. J. Cooper [5], Muaz Osman [6], S.S. Spicer [7], Partho Mukhopadhyay [8] have studied the mucosubstances of human mammary gland but few have studied about the mucin histochemical characteristics in normal human breast tissue.So the present study has been undertaken and correlated with previous workers [4-8].

Material and Methods

Histologically proven, twentyfive normal human mammary glands were obtained during autopsies and routine dissection.

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The tissue was fixed in 10% formalin with 2% Ca acetate. By routine procedure paraffin blocks were prepared and 4-5 micron thick sections were cut.

They were stained by .9-11

1. Haematoxylene and Eosin (H & E).
2. Periodic Acid- Schiff (PAS).
3. Periodic Acid- Schiff with diastase digestion (PAS-D).
4. Periodic Acid- Schiff with phenyl hydrazine (PAS-PH).
5. Alcian blue 2.5 pH (AB 2.5 pH).
6. Alcian blue 1 pH (AB 1 pH).
7. Combined AB-PAS.
8. Aldehyde fuschin (AF).
9. Combined AF-AB.

All the results were tabulated according to colour intensity into different grades ranging from + to ++++.

Colour Index

1. ++++ : Very strong positive reaction.
2. +++ : Strong positive reaction.
3. ++ : Moderate reaction.
4. + : Weak reaction.
5. No staining : Negative reaction

The slides were photographed using digital camera.

The histochemical data staining methods employed in the present work are recorded according to visually estimated intensity of staining and shades withfour plus representing strongest activity. Nomenclature applied to the mucosubstances is taken from the discussion of a proposed general terminology of histochemically recognized material [12,13]. Histochemical results requiring further description and consideration are presented here along withtheir interpretations.

Observation and Results

Sections studied showed few atrophic glands surrounded by dense connective tissue. The epithelial component shows branching duct system to form a lobule. Also are seen numerous adipose cells.The epithelial cells lining the ducts are cuboidal to columnar and exhibit interspersed lymphocytes that have entered the epithelium (Figure 1).

When the sections of mammary gland are stained with PAS stain, it was found that the ducts and lobules are stained with magenta showing the presence of PAS positive substances like carbohydrate and neutral mucins (Figure 1).

With diastase digestion the magenta colour intensity was reduced indicating the presence of non-mucinous carbohydrates like glycogen (Figure 1). PAS-PH showed decrease of colour intensity indicating presence of neutral mucins. Few cells show presence of acidic mucins also. (Figure 2). AB 2.5 pH stained dark blue which confirms the presences of both types of acidic mucins. (Figure 2). When stained with AB 1 pH very few acini are stained showing presence of very few sulfomucins (Figure 2). With AF, it showed very low colour intensity, so the presence of trace amounts ofsulfomucins is confirmed (Figure 3). With combined AB-PAS staining show varied colour intensity. Many are intensely stained with magenta colour with few blue acini.It indicates the presence of combination of acidic and neutral mucins. (Figure 3). AF-AB stained blue showing presence of sialomucins while very few are stained purple which confirms the presence ofvery few sulfomucins (Figure 3).

Table 1: Showing results of Mammary gland staining

No.	Stain	Intensity	Inference
1	H&E	-	Glands surrounded by dense connective tissue
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 acidicmucins.
9	AF-AB	Blue ++ Purple +	Confirms presence of sialomucins with trace amounts of sulfomucins.

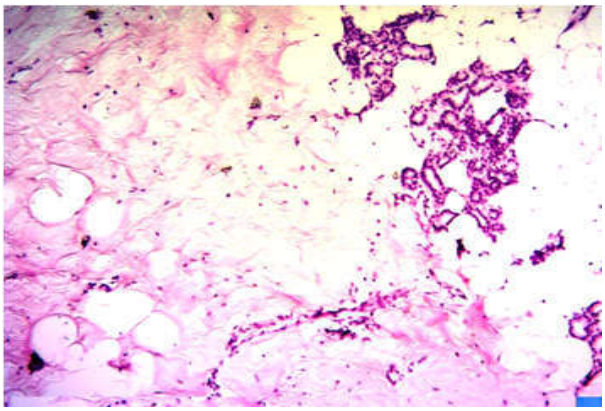

Fig. 1: Histology of normal mammary glands Inactive stage Photomicrograph 1 (H &E,10X)

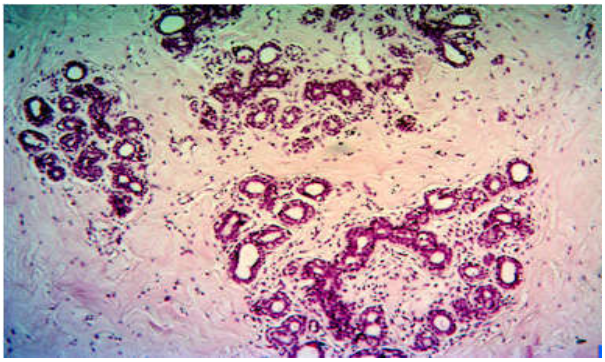

Fig. 2: PAS Photomicrograph 2 (H &E,10X)

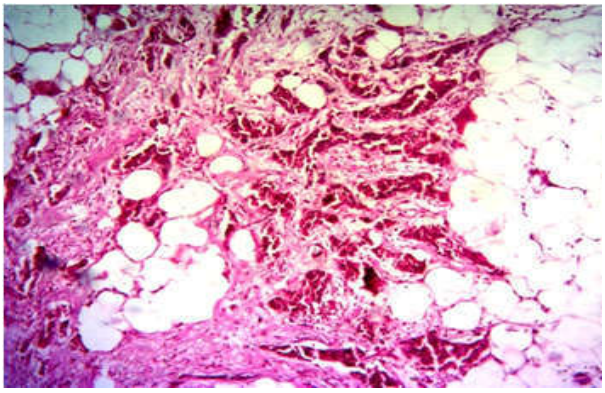

Fig. 3: PAS D Photomicrograph 3 (H &E,10X)

Fig. 1-3: Histology of normal mammary glands

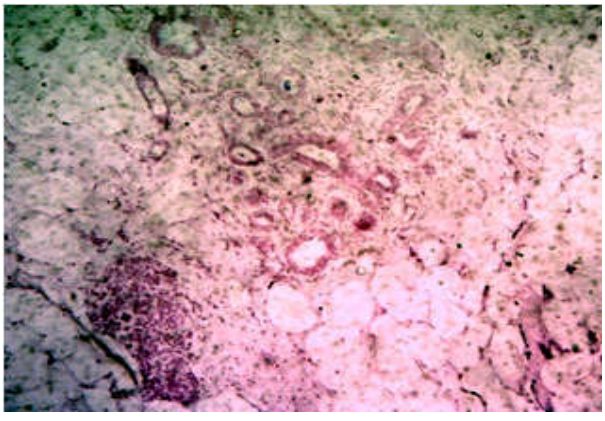

Fig. 4: PAS PH Photomicrograph 4 (H &E,10X)

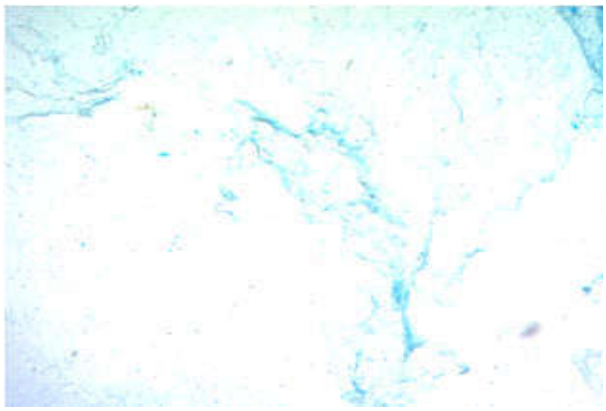

Fig. 5: AB 2.5 Photomicrograph 5 (H &E,10X)

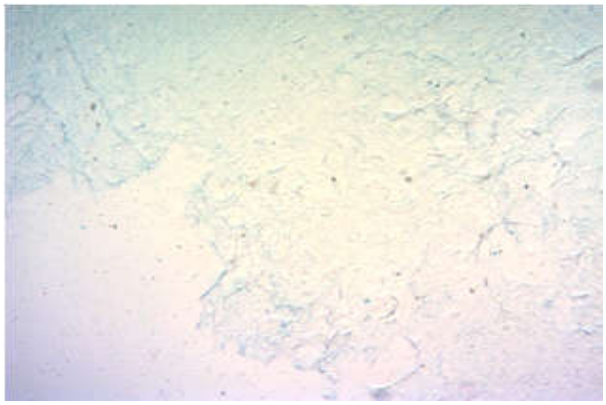

Fig. 6: PAS 1 Photomicrograph 6 (H &E,10X)

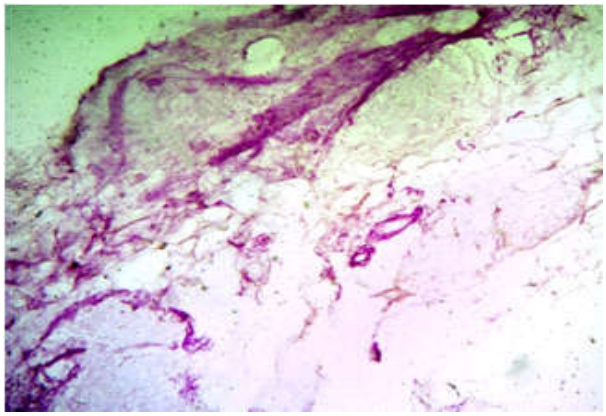

Fig. 7: AF Photomicrograph 7 (H &E,10X)

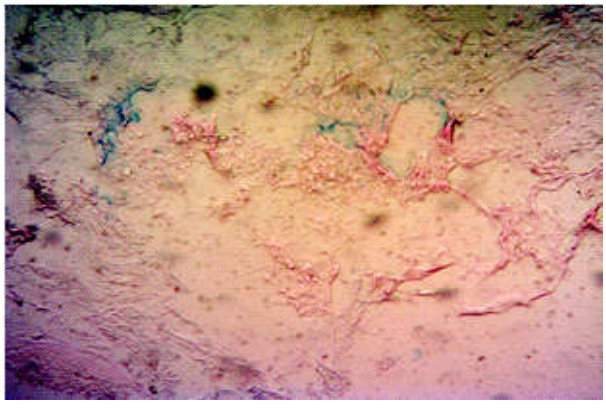

Fig. 8: AB PAS Photomicrograph 8 (H &E,10X)

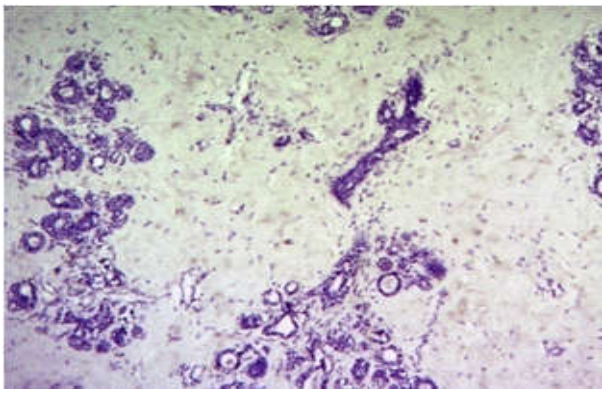

Fig. 9: AF AB Photomicrograph 9 (H &E,10X)

The results are tabulated in Table 1. From the Table 1 we can say that the mammary gland shows presence of both neutral and acidic mucins. In that also neutral are more and in acidic sialomucins are more than sulfomucins.

Discussion

Mucopolysaccharides occur in many epithelial tissues (ref 5-discussion). These substances vary greatly in chemical composition, as evidenced bythe large number of categories recognized biochemically [14] and the pluralityof their histochemical properties [15-20]. Thus neutral mucopolysaccharides can be distinguished histochemically from those containingacidic moieties [21,22]; in the latter category Inucins, in which the acidgroup is a sulfate ester [23-26], can be differentiated from those containingside groups terminating with sialic acid [27].

Displaying also a greatvariety of morphologic manifestations, mucins are found distributed uniformly throughout the cytoplasm in some glands, localized to gobletsin, various simple epithelia, restricted to superficial epithelial cells incertain stratified epithelia, and limited to the cell surface or the lumen ina number of glands.

Mucins are high molecular weight glycoproteins that are found dispersed (thesis) throughout the epithelia of the gastrointestinal, respiratory and reproductive tract [28].

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 [29]. Connective tissue mucosubstances are called "mucopolysaccharides", while those secreted by epithelia are referred as "mucins" [30].

Mucins are a family of high molecular weight, heavily glycosylated proteins (glycoconjugates) produced by epithelial tissues in most metazoans. '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 [31].

Mucins are altered in normal and pathological states so it is of ever increasing importance in the investigation of normal and disease process.

In the present study when the sections of mammary gland are stained with PAS stain, it was found that the ducts and lobules are stained with magenta showing the presence of PAS positive substances like carbohydrate and neutral mucins.

With diastase colour intensity was reduced indicating the presence of non-mucinous carbohydrates like glycogen.

PAS-PH showed presence of neutral mucins. Few cells show presence of acidic mucins also.

AB 2.5 pH confirms the presences of both types of acidic mucins. With AB 1 pH very few acini are stained showing presence of very few sulfomucins.

With AF, trace amounts of sulfomucins is confirmed. Combined AB-PAS staining indicates the presence of combination of acidic and neutral mucins.

AF-AB stained blue showing presence of sialomucins.

In normal mammary gland, the acini are positively stained with PAS indicating the presence of PAS positive substances like carbohydrates and neutral mucins. Few of the acini are negative for PAS staining which may contain enzymes or some of the sulfomucins which are PAS negative.

These results are in accordance with the study ofLuciano Ozello. Reduced magenta color intensity after diastase digestion shows the presence of non-mucinous carbohydrates also. PAS-Ph is non-reactive indicating the presence of large amount of neutral mucins.

Strong positivity for AB 2.5 but negativity to AB 1 pH and AF. It shows presence of both types of acidic mucins but in that sulfomucins are present in very much trace amounts. These results are in accordance with the study of S. S. SPICER. The combined AB-PAS and AF-AB also showed the acini positive for AB and PASindividually indicating the presence of both neutral and acidic mucins in equal amounts.

Conclusion

The human mammary gland showed the presence of neutral mucins and acidic mucins. In acidic mucins sialomucins were present and sulfomucins were recorded in trace amount. Any change in the mucin pattern may be helpful in the early diagnosis of any disease process.

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