

Fibroadenoma and Intraductal Carcinoma of Breast - A Mucin Histochemical Study

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Abstract: Background: Mucin histochemistry is one of the important branch of histochemistry aiding in identification, differentiation and understanding of the disease process. Objectives: To know the mucin distribution in fibroadenoma and intraductal carcinoma of breast. Methodology: This is a retrospective, observational, analytical, case control study aimed to evaluate mucin histochemical pattern in fibroadenoma and intraductal carcinoma of breast. Twenty five histologically proven blocks of fibroadenoma and intraductal carcinoma of breast 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 fibroadenoma; periductal stroma was PAS negative. While interlobular stroma was PAS positive and showed predominance of neutral mucins. Periductal and intralobular stroma showed predominance of acidic mucins. In Intraductal carcinoma of breast; "sialomucins" were seen predominant over neutral and sulphomucins. Conclusion: Mucin histochemical patterns serve as valuable, cost-effective tool in diagnostic histopathology and for the researchers in histology, where a slight change in the mucin pattern may help in the early diagnosis of the disease.

Keywords: Mucosubstances, Special stains, Fibroadenoma, Intraductal Carcinoma

1. Introduction

While most breast carcinomas are sporadic in nature, approximately, 5% of patients have a hereditary predisposition to develop this malignancy. Evidences are emerging, those suggest that breast carcinoma is a heterogeneous disease at the molecular level having a number of distinct entities with specific pathologic features and biologic behaviours.¹ Amongst all carcinomas of breast, Intraductal carcinoma is the commonest. Hence the approach of present study is towards comparing patterns of mucin expression in commonest occurring neoplastic breast lesions, while other rarely occurring neoplasms are not considered.

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. Nuclear changes are visible after the secretory changes occur and hence study of mucins may help to identify cancerous conditions at an early stage.² Thus early diagnosis even before carcinoma in situ will be of significant value clinically, thereby reducing the morbidity and mortality in the patients.

2. Material and Methods

The present study was conducted in the Department of Pathology and Anatomy, Krishna Institute of Medical Sciences, University, Karad from Oct 2013 to Nov 2018. The type of study was observational, analytical and case control study. Sample size was 25 blocks of histologically proven fibroadenoma and intraductal carcinoma of breast collected from surgically removed (punch biopsy) specimens from Krishna Hospital, Karad and Siddhivinayak Cancer hospital, Miraj. Staining with H and E, special stains like PAS, PAS-D, PAS-PH, AB-PAS, AB-PH2.5 and 1, AF and AF-AB was carried out and results were interpreted.

3. Observations and Results

The results were tabulated according to color intensity into different grades ranging from + to +++++.

Colour index ^{3, 4, 5}:

- 1) +++++: Very strong positive reaction.
- 2) ++++: Strong positive reaction
- 3) ++: Moderate reaction
- 4) +: Weak reaction
- 5) -: Negative reaction

Table 5: Histochemical reactions of Fibroadenoma Breast

S. No	Strain used	Color	Intensity	Duct Cells	Duct Lumen	Lobule cells	Lobule lumen	Inference about mucosubstances
1	PAS	Magenta	++	++	++	++	++	PAS positive present
2	PAS-D	Magenta	++	-	++	++	++	Glycogen absent. Mucosubstances present, except in duct cells.
3	AB pH 2.5	Dark Blue	+++	-	++	++	++	Acidic mucosubstances present in duct and lobule lumen and lobule cells. Absent in Duct cells.
4	AB pH 1.0	Faint Blue	-	-	-	-	-	Sulphated acidic mucins absent
5	AF	Weak Purple	-	-	-	-	-	Sulphated acidic mucosubstances absent
6	AB-PAS	Magenta	++	++	++	++	++	Neutral mucins present
		Blue	++	-	++	++	++	Carboxylated acidic mucins present in duct and lobule cells. Absent

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								in Duct cells
		Purple	-	-	-	-	-	Sulphated acidic mucins absent.
7	AF-AB	Blue	++	++	++	++	++	Carboxylated acidic mucins present.
		Purple	-	-	-	-	-	Sulphated acidic mucins absent
8	PAS-PH	Magenta	++	++	++	++	++	Neutral mucins present in duct and lobule lumen as well as within duct and lobule cells

Table 4: Histochemical results of Intraductal Carcinoma Breast

S. No	Strain used	Color	Intensity	Inference about mucosubstances
1	PAS	Magenta	++	PAS positive mucosubstances present
2	PAS-D	Magenta	+++	Glycogen absent. Mucosubstances present
3	AB pH 2.5	Blue	+++	Acidic mucosubstances present
4	AB pH 1	Faint Blue	-	Sulphated acidic mucins absent
5	Af	Faint Purple	+/-	Trace or absent sulphated acidic mucins.
6	AB-PAS	Magenta	+++	Neutral mucins predominant.
		Blue	++	Acidic mucins present.
		Purple	-	Sulphated acidic mucins absent.
7	AF-AB	Blue	++	Carboxylated acidic mucins present.
		Purple	-	Sulphated acidic mucins absent
8	PAS-PH	Magenta	+++	Neutral mucins predominant.

Inference

Regarding mucin histochemistry of Fibroadenoma breast, sections using PAS-D revealed periductal stroma to be PAS negative, whereas interlobular stroma was PAS positive. AB pH 2.5, confirmed the presence of acidic mucins in periductal and intralobular stroma. AB pH 1.0, AF, AB-PAS, AF-AB confirmed the absence of sulphomucins amongst acidic mucins. AB-PAS and PAS-PH showed predominance of neutral mucins in interlobular stroma.

Regarding mucin histochemistry of IDC, mixture of both neutral and acidic mucins was found. Neutral mucins were in predominance as compared to normal breast. In acidic, no sulphomucins but moderate sialomucins were seen. So the mucin pattern was shifted as compared to the normal breast.⁶

4. Discussion

Mucin is a glycoprotein bearing a high molecular weight that is synthesized, stored and secreted by connective tissue and epithelial mucosal cells, especially the goblet cells.⁷ 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 sometimes the same cell.⁸ However, under pathologic conditions this distinct expression patterns be modified.

In a high proportion of patients, benign and malignant neoplasms of the breast secrete acid mucins, the basophilia of which is attributable to sialic acid. These sialomucins resemble all the others identified histochemically by enzymatic digestion. The great variability of the histochemical properties of mammalian mucopolysaccharides.⁹⁻¹³ Suggests the possibility that identification of the type of mucosubstance secreted in a tumor might prove useful diagnostically.

In the evaluation of results, we have to take into account limitations of the techniques utilized. Fixation in formalin permits only a qualitative evaluation and not an accurate quantitative appraisal. Several investigators have suggested that breast stroma contains acid mucopolysaccharides¹⁴⁻¹⁸ the

basis of metachromasia which is totally or partially eliminated by hyaluronidase.

5. Summary and Conclusion

Mucin histochemistry of fibroadenoma and Intraductal Carcinoma of breast was undertaken in the department of Pathology and Anatomy at Krishna Institute of Medical Sciences University, Karad from Oct 2013 to Nov 2018. Haematoxylin and Eosin was used as routine stain for identification of tissue and confirmation of diagnosis.

- Special stains such as PAS, PAS-Diastase, and PAS-Phenyl hydrazine were used for confirmation of neutral mucins.
- Alcian blue pH 2.5 was used to assess acidic mucin. Further categorization of acidic mucins into sulphomucins (sulphated) and sialomucins (carboxylated) was carried out by Alcian blue pH 1.0 and Aldehyde fuchsin.
- Combined stains such as AB-PAS, AF-AB and PAS-Phenyl hydrazine were used to differentiate between neutral and acidic mucins.
- Mucin histochemistry of Fibroadenoma showed that neutral as well as acidic mucins were present. In acidic, sialomucins were present and sulfomucins were absent.
- Histochemical results for IDC breast showed a mixture of both neutral as well as acidic mucins, with predominance of neutral mucins. In acidic, moderate amounts of carboxylated mucins (sialomucins) were seen and sulphated mucins (sulphomucins) were absent, which correlated well with various workers.

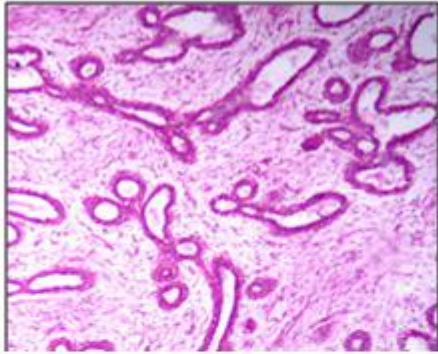
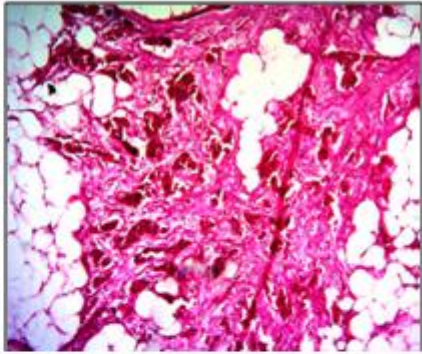
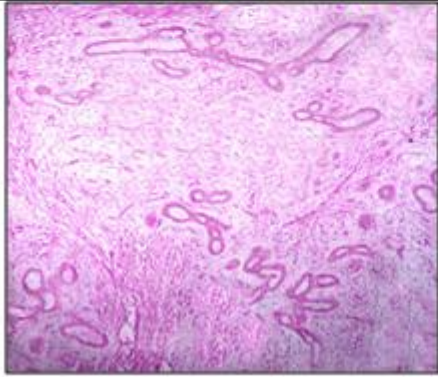
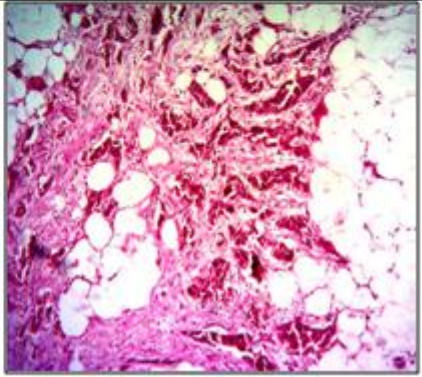
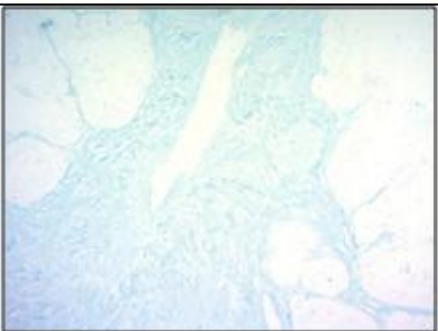
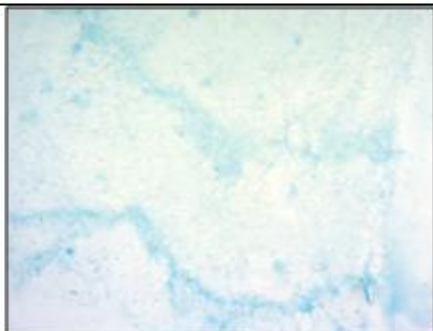
6. Perspectives and Recommendations

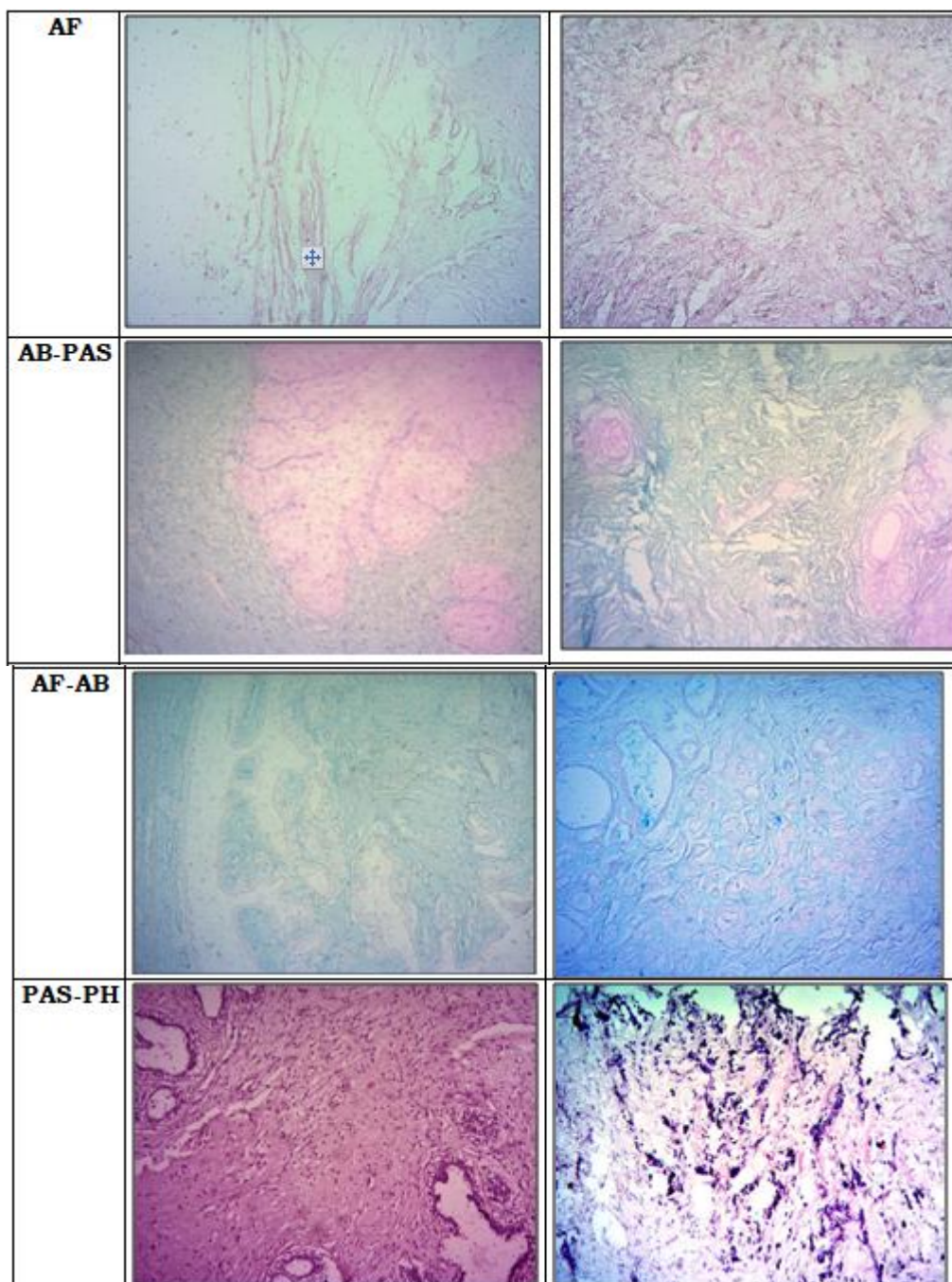
Breast cancer ranks second amongst all the causes of cancer deaths in females and is a problem worldwide. In spite of all efforts taken to identify factors which are responsible for aggressiveness of this disease, the exact succession of molecular events that has led to the development of this devastating disease has remained unclear. Patterns of mucin histochemistry can play valuable, cost-effective and important role in the diagnosis of IDC breast. Mucins have

emerged as important molecules in the progression and metastasis of IDC breast. Changes in their expression, glycosylation and presence of multiple splice variants are currently under active investigation to better understand their role in BC pathogenesis.

6.1 Recommendations

Current and emerging evidences suggest that mucins are differentially expressed during progression and metastasis of BC, and thus, its recommended that histochemical study of mucin could be extremely useful in either early detection or predicting prognosis, or both, of BC patients. It's recommended to accompany Immune-expression tests of mucin gene which will be more valuable and helpful diagnostic tool and it can elucidate the patho-physiological significance of mucin in BC.

Stain	Fibroadenoma	IDC
PAS		
PAS-D		
AB pH 2.5		
AB pH1		



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