



## COMPARISON OF HISTOCHEMICAL CHARACTERISTICS BETWEEN ADENOCARCINOMA OF ENDOCERVICAL GLANDS AND INTRADUCTAL CARCINOMA OF BREAST

**Dr. Ajay J. Shedde** Assosiate Professor, Dept of Patholgy, AIMS, Dewas, MP

**Dr. Shedde Swapna A\*** Assistant Professor Department Of Anatomy, KIMS, Deemed To Be University, Karad, Maharashtra . \*Corresponding Author

**ABSTRACT** **BACKGROUND:** 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. Mucins are mainly of two types- Neutral and Acidic. Mucus plays an important role in reproductive function and defense of the female reproductive tract.

**OBJECTIVES:** To know and compare the mucin distribution of the adenocarcinoma of endocervical glands and intraductal carcinoma of breast.

**METHODOLOGY:** This is a retrospective, observational, analytical, case control study aimed to evaluate mucins histochemical pattern in adenocarcinoma of endocervical glands and intraductal carcinoma of breast.

Twenty five histologically proven blocks of adenocarcinoma of endocervical glands 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 adenocarcinoma of endocervical glands; a mixture of both neutral and acidic mucins are found. Neutral mucins are in trace amounts. In acidic, no sialomucins but few sulphomucins are seen. 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, Adenoarcinoma, Intraductal Carcinoma.

### INTRODUCTION

The mucins are high molecular weight glycoproteins in which proline and threonine constitute up to 20-55% of total amino acids and are concentrated in one or several regions of the polypeptides. The mucosubstances are complex molecules which contain both proteins and polysaccharide components linked together with strong covalent chemical bonds. They are conveniently divided into glycoproteins and proteoglycans on the basis of structural characteristics.<sup>1</sup>

Epithelial mucins may be present as mixture of various types which have been classified as neutral and acidic.<sup>2</sup> The mucins are principal organic constituents of mucus. The slimy visco-elastic material that envelopes all mucosal surfaces of the body. This adherent layer consists of proteins, lipids and glycoproteins forming a jelly like coating that serves as a protective cover against microbial, mechanical and chemical damage to the underlying tissues.<sup>3</sup> All mucins share certain common structural features, but are distinct in the sequence, domain organization, length, and number of their respective tandem repeat sequences.<sup>4</sup>

### FUNCTIONS OF MUCIN

1. Functional studies have also exposed many significant roles of mucins during the progression of breast cancer, which include modulation in proliferative, invasive and metastatic potential of tumour cells.<sup>5</sup>
2. Mucins function through their property of forming complex association with various signalling molecules including growth factor receptors and intercellular adhesion molecules.<sup>3</sup>
3. Mucins key characteristic is their ability to form gels; therefore they are a key component in most gel-like secretions. Thus serving functions from forming chemical barriers, lubrication and cell signaling. They often take an inhibitory role.<sup>7</sup>
4. Some mucins are associated with controlling mineralization, including nacre formation in mollusks,<sup>8</sup> calcification in echinoderms and bone formation in vertebrates.<sup>10</sup>
5. The mucins maintain adequate epithelial hydration thus preventing tissue oedema.<sup>11,12</sup>
6. Sialic acid also exhibits a viscoelastic property.<sup>13</sup>
7. Sulfo mucins by forming a jelly like blanket, protect the mucosal surfaces thus exhibiting an antiulcerogenic property. The mucins play an important role in defense against bacteria.<sup>14</sup>
8. The mucosubstances also contain immunoglobulins primarily of

IgA type, lactoferrin which chelate the iron necessary for growth of some bacteria and lysozymes which destroy some of the bacteria. Hence they act as antibacterial and antiviral agents and have protective mechanism.<sup>15</sup>

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.<sup>15</sup> Thus early diagnosis even before carcinoma in situ will be of significant value clinically, thereby reducing the morbidity and mortality in the patients.

### MATERIAL AND METHODS

The present study was conducted in the Department of Pathology, Krishna Institute of Medical Sciences, University, Karad from May 2010 to June 2012 and 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 adenocarcinoma of endocervical glands and intraductal carcinoma of breast were taken. Work protocol was submitted to the protocol and ethical committee for approval and necessary permission was taken. The study was undertaken on 25 specimens of malignant endocervical glands 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.

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 embedded in paraffin 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 such 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.

### OBSERVATIONS AND RESULTS

The results were tabulated according to color intensity into different

grades ranging from + to +++++.<sup>12,13,14</sup>

**Colour index 12,13,14 :**

1) +++++ : Very strong positive reaction.

2) +++ : Strong positive reaction

3) ++ : Moderate reaction

4) + : Weak reaction

5) - : Negative reaction

**Table showing histochemical results of adenocarcinoma endocervical glands and Intraductal Carcinoma Breast .**

Sr. No.	Stains used	Inference about mucosubstances	
		Adenocarcinoma endocervix.	Intraductal Carcinoma Breast
1	H & E	Adenocarcinoma identified and confirmed. Photomicrograph I (H &E)	Intraductal Carcinoma identified and confirmed. Photomicrograph II (H &E)
2	PAS	PAS +ve substances present Photomicrograph 1a (PAS 10X)	PAS +ve substances present. Photomicrograph 1a (PAS 10X)
3	PAS- Diastase.	No glycogen. Photomicrograph 2a (PAS-D 10X)	No glycogen. Photomicrograph 2a (PAS-D 10X)
4	PAS- PH	Trace neutral mucins. Photomicrograph 3a (PAS-PH 10X)	Neutral mucins predominant. Photomicrograph 3a (PAS-PH 10X)
5	AB-pH 2.5	Trace amount of acidic mucins. Photomicrograph 4a (AB PH 2.5 10x)	Acidic mucosubstances present.. Photomicrograph 4a (AB PH 2.5 10x)
6	AB – pH1	No Sulphomucins. Photomicrograph 7a (AB 1 -10X)	Sulphated acidic mucins absent. Photomicrograph 7a (AB 1 -10X)
7	AB- PAS	Trace amount of acidic mucosubstances. Photomicrograph 6a (AB-PAS 10X)	Neutral mucins predominant. Acidic mucins present. Sulphated acidic mucins absent.. Photomicrograph 6a (AB-PAS 10X)
8	AF	Trace or no amount of sulphomucins. Photomicrograph 5a (AF 10X)	Trace or absent Sulphated acidic mucins.. Photomicrograph 5a (AF 10X)
9	AF-AB	Trace amount of sulphomucins. Photomicrograph 8a (AF-AB 10X)	Carboxylated acidic mucins present. Sulphated acidic mucins absent.. Photomicrograph 8a (AF-AB 10X)

**INFERENCE :**

Regarding mucin histochemistry of adenocarcinoma endocervix, very few mucins are present. A mixture of both neutral and acidic are found. Neutral mucins are in trace amounts. In acidic, no sialomucins but few sulphomucins are seen. So there is a shift in mucin pattern as compared to normal.<sup>16</sup> Neutral mucins are almost seen to be disappearing. Sulphomucins alone are in trace amounts. Regarding mucin histochemistry of IDC, mixture of both neutral and acidic mucins were 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.<sup>17</sup>

**DISCUSSION**

Worldwide, cervical cancer is the fifth most deadly cancer in women.<sup>18</sup> It affects about 16 per 100,000 women per year.<sup>19</sup> It is most important and accounts for 20-25% of all the cancers and 85% of all the female genital tract malignancies. Primary adenocarcinoma make 5-10% of all cancers of the cervix.<sup>20</sup> The amount of stainable mucin may show marked variation in malignant lesion as compared to normal endocervical glands.<sup>21</sup>

Invasive breast cancer is the most common carcinoma in women, accounting for 23% of all cancers in women globally<sup>22</sup> and now the most common cancer in Indian women, having recently overtaken cervical cancer in this respect.<sup>23</sup> After lung cancer, breast cancer (BC) is the next commonest cause of deaths in women all over the world. In the year 2010, BC accounted for an estimated 28% of all new cancer cases in the United States, while nearly 15% deaths from this malignancy occurred in the same period.<sup>4</sup>

Identification, differentiation and understanding of the disease process is the sole motive of mucin histochemistry. Nowadays immunohistochemistry, autoradiography and genetic studies are replacing it. But all these diagnostic modalities are costly and cannot be fulfilled without well-equipped laboratories and trained personnel. Neither are they available at rural or secondary health care centres. Hence the first choice of investigation is mucin histochemistry.

**SUMMARY AND CONCLUSION**

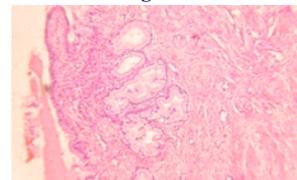
Mucin histochemistry of adenocarcinoma endocervical glands and Intraductal Carcinoma of breast was undertaken in the department of Pathology at Krishna Institute of Medical Sciences University, Karad from May 2010 to June 2012 and from Oct 2013 to Nov 2018.

- 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 sulpho and sialomucins was

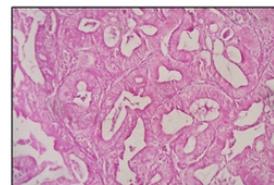
carried out by Alcian blue pH 1 and Aldehyde fuchsin.

- Combined stains such as AB-PAS, AF-AB and PAS-Phenyl hydrazine were used to differentiate between neutral and acidic mucins.
- Histochemical results for malignant endocervical glands showed very few mucins. Mixture of both neutral and acidic were found. Neutral mucins were in trace amounts. In acidic, no sialomucins but few sulphomucins were seen. So there is a shift in mucin pattern as compared to normal. Neutral mucins were almost disappearing. Sulphomucins alone were in trace amounts.
- 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 absence of sulphated mucins (sulphomucins), which correlated well with various workers.
- Mucin content and type of mucin present can serve as an important prognostic indicator and early diagnosis may help in reducing the mortality related to endocervical malignancies.

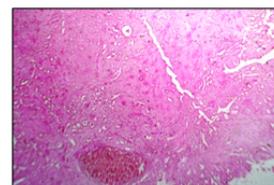
**Adenocarcinoma endocervical glands**



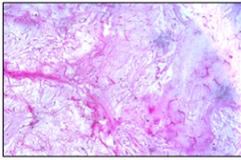
**Photomicrograph I (H & E, 10X)**



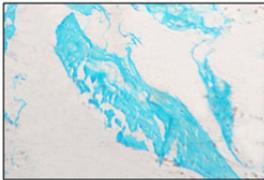
**Photomicrograph 1 (PAS 10X)**



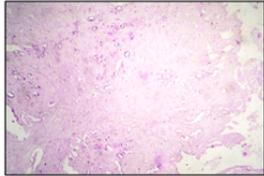
**Photomicrograph 2 (PAS-D 10X)**



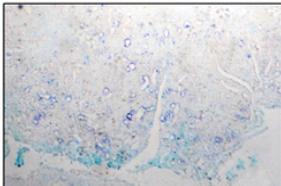
Photomicrograph 3 (PAS-PH 10X)



Photomicrograph 4 (AB PH 2.5 10X)



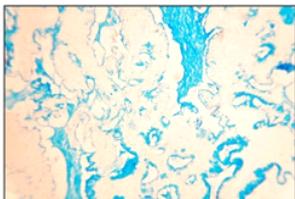
Photomicrograph 7 (AF-10X)



Photomicrograph 6 (AB-PAS 10X)

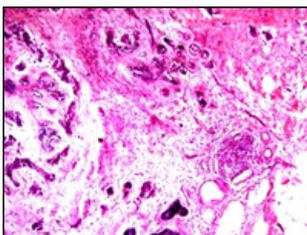


Photomicrograph 5 (AB 1-10X)

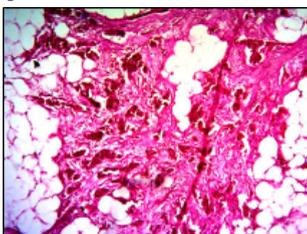


Photomicrograph 8 (AF-AB 10X)

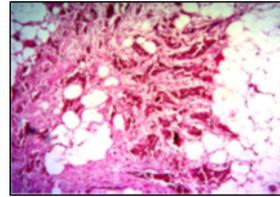
**Intraductal Carcinoma of breast**



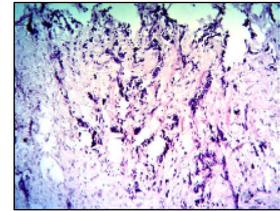
Photomicrograph II H&E



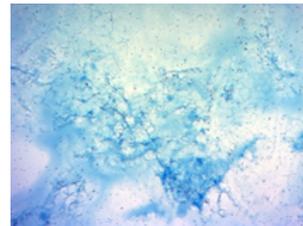
Photomicrograph 1a Periodic Acid-Schiff (PAS). (10X)



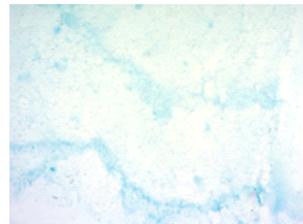
Photomicrograph 2a Periodic Acid-Schiff with diastase digestion (PAS-D). (10X)



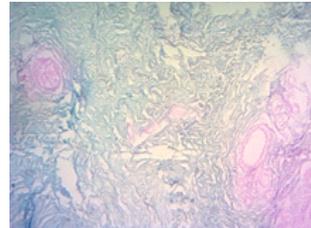
Photomicrograph 23 Periodic Acid-Schiff with phenylhydrazine (PAS-PH). (10X)



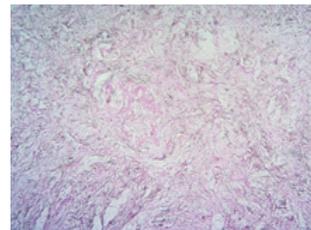
Photomicrograph 3a Alcian blue pH 2.5 (AB pH 2.5). (10X)



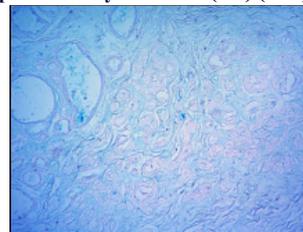
Photomicrograph 5a Alcian blue pH 1.0 (AB pH 1.0). (10X)



Photomicrograph 6a Periodic Acid-Schiff with Alcian blue (AB-PAS). (10X)



Photomicrograph 7a Aldehyde fuchsin (AF). (10X)



Photomicrograph 8a Combined Aldehyde fuchsin - Alcian blue (AF-AB). (10X)

## REFERENCES

1. Naag S. and Adi R. P. Histochemical study of salivary mucins in normal and neoplastic salivary glands. *Journal of clinical and diagnostic research* 2010 December; 4:3450-3458.
2. Barrett AJ. The biochemistry and function of mucosubstances. *Histochemical Journal* 1971; p213-221.
3. Filipe, M. I., and Branfoot, A. C. Mucin histochemistry of the colon. *Current Topics in Pathology*; 1976, p 143-178.
4. Partha Mukhopadhyay, Subhankar Chakraborty, Moorthy P. Ponnusamy, Imayavaramban Lakshmanan, Maneesh Jain, and Surinder K. Batra. Mucins in the Pathogenesis of Breast Cancer: Implications in Diagnosis, Prognosis and Therapy. *Biochim Biophys Acta* . 2011 April ; 1815(2): 224–240. doi:10.1016/j.bbcan.2011.01.001.
5. Ghoncheh M, Momenimovahed Z, Salehiniya H. Epidemiology, incidence and mortality of breast cancer in Asia. *Asian Pac J Cancer Prev*. 2016;17:47-52.
6. Marin, F. D. R.; Luquet, G.; Marie, B.; Medakovic, D. (2007). Molluscan Shell Proteins: Primary Structure, Origin, and Evolution. *Current Topics in Developmental Biology* 80. p. 209. doi:10.1016/S0070-2153(07)80006-8. ISBN 9780123739148.
7. Marin, F.; Corstjens, P.; De Gaulejac, B.; De Vrind-De Jong, E.; Westbroek, P. (2000). "Mucins and molluscan calcification. Molecular characterization of mucoperlin, a novel mucin-like protein from the nacreous shell layer of the fan mussel *Pinna nobilis* (Bivalvia, pteriomorpha)". *The Journal of Biological Chemistry* 275 (27): 20667–20675. doi:10.1074/jbc.M003006200. PMID 10770949.
8. Boskey, A. (2003). "Biom mineralization: an Overview". *Connective Tissue Research* 44 (1): 5–9. doi:10.1080/713713622. PMID 12952166.
9. R J Midura, V C Hascall (1996). "Bone sialoprotein—a mucin in disguise?". *Glycobiology* 6 (7): 677–81. doi:10.1093/glycob/6.7.677. PMID 8953277.
10. Taylor JJ and Flaa RC. Histochemical analysis of Paneth cell granules in rats. *Arch Path (Chicago)*, 1974; 77:278.
11. Kent P. W. Biosynthesis of intestinal glycoproteins in animals and man. *Gut*. 1971, 12: 417.
12. Traynor OJ, Wood CB and Costa N. Ultrastructural Alterations in the Colonic Mucus Layer During Carcinogenesis : A Scanning Electron Microscopy Study.(ed. M. Elastin and DV Parke) London Plenum press NYP 1982. P-225-29.
13. Forstner G, Welsy A, Forstner J. Clinical aspect of gastrointestinal mucous in health and disease II' Elastin M and Parke, D. V. eds, Plenus Press N. Y., P-199-225, 1981.
14. Alan stevans. The haematoxylin in: Bancroft JD, Stevans A (editors). *Theory and practice of histological technique*. 3 rd ed. Churchill livingstone, New York. 1990; 107-117.
15. Hartly, W.G. 1993. *The Light Microscope. : Its use and development* Scenario Publishing Co., Oxford (1993) ISBN 0–906831–05–9; Senecio Publishing Company. Oxford; 1,3,4.
16. Dr. Ajay J. Shedge: Histochemical Characteristics Of Mucosubstances In Adenocarcinoma Of Endocervical Glands. *IJSR*, Volume-8 | Issue-4 | April-2019 | PRINT ISSN No 2277 - 8179
17. Dr. Shedge Swapna A. Histochemical Characteristics of Mucosubstances in Diseased Breast Indian Journal of Anatomy Volume 7 Number 3, May - June 2018 ,pg 285-290, DOI: <http://dx.doi.org/10.21088/ija.2320.0022.7318.11>
18. World Health Organization (Feb.2006), "Fact Sheet No.297: Cancer". Retrieved; 2007-12-01.
19. GLOBOCAN 2002 Database: "Summary table by Cancer". Retrived 2008-10-26.
20. Carcinoma of the cervix in Shaw's text book of Gynaecology, 10th edition, Ed. Padulidri V., Daftary SN, 428-460, 1989
21. Lauchlan SC: Metaplasia and neoplasias of mullerian epithelium. *Histopathology* 8 : 543-57, 1984).
22. Colditz G, Chia K.S. Invasive breast carcinoma: Introduction and general features. In: Lakhani SR, Ellis IO, Schnitt SJ, Tan PH, Van de Vijver MJ. *WHO Classification of tumours of the breast*, 4th ed, Lyon: IARC; 2012. pg 14.
23. Ghoncheh M, Momenimovahed Z, Salehiniya H. Epidemiology, incidence and mortality of breast cancer in Asia. *Asian Pac J Cancer Prev*. 2016;17:47-52.