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HISTOCHEMICAL CHARACTERISTICS OF MUCOSUBSTANCES IN ADENOCARCINOMA OF ENDOCERVICAL GLANDS

Pathology

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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 endocervical glands in malignant lesions.

METHODOLOGY: This is a retrospective, observational , analytical, case control study aimed to evaluate mucins histo-chemical pattern in malignant endocervical glands. Twenty five histologically proven adenocarcinoma of endocervical glands 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.

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 amount. In acidic no sialomucins but few sulphomucins are seen.

CONCLUSION: Mucin histochemical patterns serve as valuable, cost-effective tool for the 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 process.

KEYWORDS

Mucosubstances, Special stains , Cervical mucins , Adenoarcinoma

INTRODUCTION

Mucins are complex carbohydrates secreted by different types of epithelial cells and glandular tissues of gastrointestinal tract. There has been growing recognition in recent years that the demonstration of these substances is difficult, complex and affected by the types of mucins present. [1,2] . Mucus plays an important role in reproductive function and defense of the female reproductive tract.[3] Cervical mucus, reported to be secreted at a rate of 20-60 mg/day, [3,4] provides a barrier to sperm and pathogen entrance into the endometrium and provides a protective covering for the vaginal epithelium. Just before ovulation, mucus character changes from a viscous to a watery consistency to allow sperms to penetrate into the uterus. Alterations in mucus quantity and quality are related to hormone/reproductive status changes, infections, and pathology of the female reproductive tract.[3,4]

The term „mucosubstances" is used, as recommended by Spicer, Leppi and Stoward [3] to denote all tissue components other than glycogen, rich in carbohydrates, which are present in connective tissue, or as secretion of certain epithelial structures. Connective tissue mucosubstances are called "mucopolysaccharides", while those secreted by epithelia are referred as "mucins". [5]

The function of mucins varies 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.[6]

Mucins perform a wide variety of functions like lubrication, protection against acids etc. The mucosubstances also contain immunoglobulins primarily of IgA type, lactoferrin 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.[7]

Mucins are classified into two main categories namely,

A) Neutral mucins,

B) 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.[8,9,10] 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.[9,10]

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.[11] Thus early diagnosis even before carcinoma in situ will be of great clinical value in reducing the morbidity and mortality in the patients..

With the development of new histochemical methods with special stains, specific chemical composition of mucosubstances is documented by various scientists, but there have been very few studies on human endocervical mucins like JV Bulmer et al (1988), V. Misra et al (1997), Zhao Shumei et al (2003), Hayashi et al (2003).

In the present study, a combination of special stains such as P.A.S.Diastase, P.A.S.- Phenyl hydrazine, Alcian Blue-P.A.S. Aldehyde fuschin-Alcian blue were used to simultaneously assess the proportions of various mucin types in the epithelium of endocervical glands examined.

MATERIAL AND METHODS

The present study was conducted in the Department of Anatomy, Krishna Institute of Medical Sciences, University, Karad from May 2010 to June 2012.

The type of study was observational, analytical and case control study. Sample size was 25 blocks of histologically proven adenocarcinoma of endocervical glands. 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 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.

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 ++++. [12,13,14]

OBSREVATIONS AND RESULTS During the period of May 2009 to June 2011, thirty blocks of normal and carcinoma colon were collected. Normal colonic specimens were used as controls with carcinoma colon as test. Histological technique was preceded and staining with H and E and with special stains as PAS, PAS-Diastase, AB-pH 2.5 and 1, AF, combined AB-PAS, AF-AB, PAS-Phenyl hydrazine were carried out.

All the results were tabulated according to color intensity into different grades ranging from + to ++++. [12,13,14]

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 malignant endocervical glands.

Sr. No.	Stain used	Result	Inference about mucosubstance
1	H & E	Adenocarcinoma identified and confirmed	-
2	PAS	++	PAS +ve substances present.
3	PAS- D	++	No glycogen,mucosubstances present.
4	PAS- PH	+	Trace neutral mucosubstances.
5	AB pH 2.5	+ / -	Trace amount of acidic mucosubstances.
6	AB pH 1	-	No Sulphomucins.
7	AB- PAS	Blue +/-	Trace amount of acidic mucosubstances.
8	AF	+ / -	Trace or no amount of sulphomucins.
9	AF - AB	Purple + / -	Trace or few amount of sulphomucins.

INFERENCE : Regarding mucin histochemistry of malignant endocervical glands, very few mucins are present. A mixture of both neutral and acidic are found. Neutral mucins are in trace amount. In acidic no sialomucins but few sulphomucins are seen. Neutral mucins are almost disappearing. Sulphomucins alone are in trace amount.

In the present study, H and E and special stains were used for mucin histochemistry of 25 specimens of adenocarcinoma cervix. Haematoxylin and Eosin was used to identify and confirm the diagnosis of adenocarcinoma. Adenocarcinoma showed abnormal, irregular and closely packed glands lined by tall columnar epithelial cells exhibiting considerable pleomorphism (Photomicrograph No.1). Regarding special stains, PAS stain was used to asses presence of neutral mucosubstances. PAS stain for adenocarcinoma cervix gave mild reaction as focal magenta staining, suggestive of presence of few neutral mucosubstances to reduced neutral mucosubstances as compared to normal endocervix (Photomicrograph No.2).

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 (Photomicrograph No. 3).

PAS-Phenyl hydrazine in adenocarcinoma cervix 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 (Photomicrograph No. 4).

For acidic mucin, in all adenocarcinomas AB-pH 2.5 carried out gave mild reaction as blue colour (Photomicrograph No.5). This is suggestive of presence trace amont of acidic mucosubstances.

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 sulphated mucins (Photomicrograph No. 6).

Combine stain AB-PAS was done to assess all different types of mucins, mainly neutral and acidic. AB-PAS stain gave weak reaction as blue suggestive of trace amount of acidic mucosubstances (Phomicrograph No. 7).

Aldehyde fuchsin stains only sulphomucins and confirms their presence and hence was carried out on 10 specimens of adenocarcinomas. AF gave weak reactions as faint purple color suggestive of presence of few sulphated mucins and compared to normal showed reduced or loss of sulphation(Photomicrograph No.8).

AF-AB technique helps to differentiate and confirm carboxylated and sulfated mucins. AF-AB on all 10 specimens of adenocarcinoma cervix gave strong reaction as blue and weak reaction as purple colour suggestive of predominance of carboxylated mucins and traces of sulphated mucins (Photomicrograph No. 9).

DISCUSSION

Worldwide, cervical cancer is the fifth most deadly cancer in women.[15] It affects about 16 per 100,000 women per year.[16] 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[17]. The amount of stainable mucin may show marked variation in malignant lesion as compared to normal endocervical glands.[18] Benda et al[23] were the first to demonstrate the importance of mucin secretion in cervical cancer which was confirmed later on.[19,20,21] Broadly cervical cancer is categorized into squamous cell carcinoma, adenocarcinoma and mixed carcinoma. However lesion diagnosed as moderately or poorly differentiated squamous cell carcinomas on H & E stain may turn out to be squamous cell carcinoma with mucin secretion, adenosquamous carcinoma or adenocarcinoma after staining with mucin stains depending upon the amount of mucin present. This emphasizes the importance of mucin stain as a routine for diagnosis of cervical carcinoma.[22]

Comparative study of Adenocarcinoma of endocervix

Lapertosa G, Baracchini P et al studied 20 specimens of normal endocervix and 10 of adenocarinomas. In adenocarcinomas, mucin secretion was usually scanty and mixed with a predominance of sialomucins.[23]

J.V. Bulmer, N.R. Griffin, C. Bates studied 2 cases of adenocarcinoma endocervix and found that both cases contained abundant neutral mucins and sialomucins, whereas sulfomucins were rarely detected.[24] Vatsala Misra, et al re-classified carcinoma cervix uteri by mucin histochemistry with 6 cases of adenocarcinomas and 12 normal. In malignant lesions, a marked decrease in neutral mucins with a corresponding increase in sulfomucins was the predominant pattern. Sialomucins were also marginally increased.[25]

Hayashi, Isamu M.T., Tsuda Hiroshi studied the diagnosis of Minimal Deviation Adenocarcinoma (MDA) of the cervix by histochemistry. In all 11 MDAs and 11 (out of 41) conventional cervical adenocarcinomas the tumour cell cytoplasm was stained diffusely red by PAS indicating exclusive production of neutral mucins. The amount of acid mucins, both sulfo and sialomucins was decreased by HID-AB. Of 30 conventional cervical adenocarcinomas 28 showed acid and neutral mucins and 2 showed acid mucins only in goblet cells or in part of the cytoplasm or cell surface of constituent cells, where acid mucins consisted predominantly sulfomucins in 14 and sialomucin in 16.[26]

SK Mathur, et al studied the significance of mucin secretion in carcinoma of uterine cervix. They concluded that mucin staining should be done in all cases of carcinoma cervix in order to avoid errors in diagnosis and to detect poorly differentiated mixed carcinomas, which may escape detection on H and E staining alone.[27]

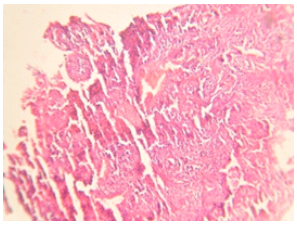
Table showing comparative study of mucin histochemistry of adenocarcinoma endocervix.

Sr. No.	Worker	Inference/Observation.
1	Lapertosa G et al[23](1986)	Mucin secretion scanty, mixed with predominance of sialomucins.
2	JV Bulmer et al [28] (1988)	Abundant neutral mucins and sialomucins. Sulphomucins rarely detected.
3	V.Misra et al[25] (1997)	Marked increase in neutral mucins with corresponding increase in sulphomucins. Sialomucins marginally increased.
4	Hayashi et al[26] (2003)	Mixture of neutral and acidic mucins. Acid mucins (sulphomucins and sialomucins) decreased. Sialomucins marginally increased.
5	Present study	Mucin secretion scanty. Mixture of neutral and acidic mucins. Neutral mucins in trace amount. In acidic, no sialomucins but few sulphomucins.

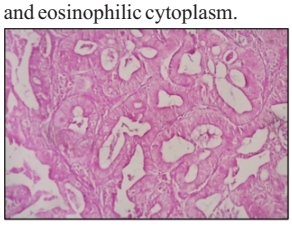
SUMMARY AND CONCLUSIONS

- In the present study, routine and special histochemical methods were applied for Adenocarcinoma of endocervical glands. H &E used as routine stain for the identification of tissue and diagnosis of carcinoma.
- Special stains as PAS and PAS-diastase, 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, PAS-phenyl hydrazine were used to differentiate between neural and acidic mucins. The varied heterogenecity of acidic mucins was indicated by a mixture of sulpho and sialomucins.
- Histochemical results for malignant endocervical glands showed very few mucins. Mixture of both neutral and acidic were found. Neutral mucins were in trace amount. In acidic, no sialomucins but few sulphomucins were seen.Neutral mucins were almost disappearing. Sulphomucins alone were in trace amount.
- Reduction in amount or absence of neutral mucins in malignant lesion with corresponding increase in sulphomucin correlated well with various workers.

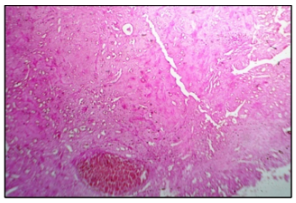
Histology of Adenocarcinoma endocervix



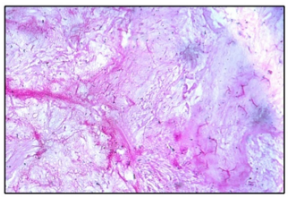
Photomicrograph 1 (Haematoxylene and Eosin 10X)
Section shows a tumour composed of abnormal, irregular and closely packed glands lined by tall columnar cells exhibiting considerable nuclear pleomorphism. Also are seen sheets of cells with ovoid nuclei and eosinophilic cytoplasm.



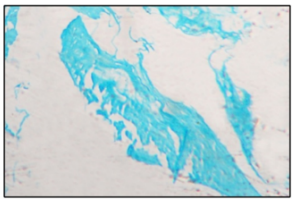
Photomicrograph 2 (PAS 10X)



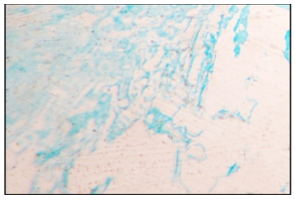
Photomicrograph 3 (PAS-D 10X)



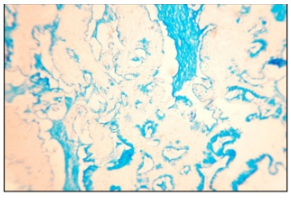
Photomicrograph 4 (PAS-PH 10X)



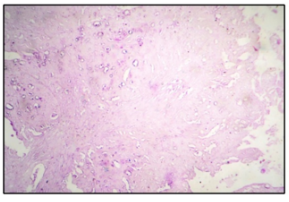
Photomicrograph 5 (AB PH 2.5 10X)



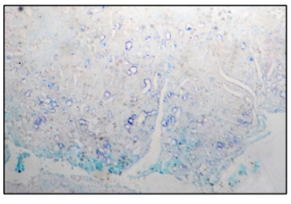
Photomicrograph 6 (AB 1 10X)



Photomicrograph 7 (AB-PAS 10X)



Photomicrograph 8 (AF 10X)



Photomicrograph 9 (AF-AB 10X)

REFERENCES

1. Stanforth DA. Staining methods: Carbohydrates and amyloid. In Wulff S. (edi), Guide to special stains. 1st ed. California: Dako; 2004:48-49.
2. Filipe MI, Branfoot AC. Abnormal patterns of mucous secretion in apparently normal mucosa large intestine with carcinoma. Cancer.1974; 34: 282-290.
3. Hafez ESE. Human Reproduction. Conception and Contraception. Hagerstown: Harper and Row; 1980: 221-252.
4. Blandau RJ, Moghissi K. The Biology of the Cervix. Chicago: The University of Chicago Press; 1973: 450.
5. Drury RAB, Wallington EA. The theory and practice of staining in Carleton"s histological technique. 5th ed. Oxford University Press, Great Britain.1980: 107-118.
6. Daunter B, Counsilman C. Cervical mucus: its structure and possible biological functions. 1980 Mar;10(3):141-61.
7. 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.
8. Stanforth DA. Staining methods :Carbohydrates and Amyloid. In Wulff S. (edi), Guide to special stains. 1st edition. California: Dako; 2004:48-49.
9. Drury RAB, Wallington EA. The theory and practice of staining in Carleton's histological technique. 5th ed. Oxford University Press, Great Britain. 1980 : 107-118.
10. Alan Stevans. The Haematoxylin in : Bancroft JD, Stevans A (editors). Theory and practice of histological technique.3rd ed. Churchill Livingstone, New York. 1990:107-117.
11. Hadi NI Kazi AS, Bushera W. Is mucin content a prognostic indicator in colorectal carcinoma. Jr. of surgery pakistan (International).2009;14:7-10.
12. Gad A. A histochemical study of human alimentary tract mucosubstances in health and disease: Normal and tumors. British Jr Cancer. 1969; 23(1): 52-63.
13. Tock EP and Tan NT. A histochemical study of the mucins of the adult human nasopharynx. J. Anat. 1969; 104(1): 81-92.
14. Taib Noory T and Jarrar Bashir M. Histological and histochemical characterization of the lingual saliavary glands of the Quil,Coturnix Coturnix. Saudi J. Bio. Sci. 1998; 5(2):33-40.
15. World Health Organization (Feb.2006), "Fact Sheet No.297: Cancer". Retrieved; 2007-12-01.
16. GLOBOCAN 2002 Database: "Summary table by Cancer". Retrived 2008-10-26.
17. Carcinoma of the cervix in Shaw's text book of Gynaecology,10th edition, Ed. Padulidri V., Daftary SN,428-460,1989)
18. Lauchlan SC: Metaplasia and neoplasias of mullerian epithelium. Histopathology 8 : 543-57,1984).
19. Kent A. "HPV Vaccination and Testing." Review in Obstretics & Gynaecol 2010; 3(1); 33-34.

20. Buckley CH, Beards CS, Fox H. Pathologic prognostic indicators in cervical cancer with particular reference to patients under the age of 40yrs. Br J. Obstretic. Gynaecol 1988; 95; 47-56.
21. Bethwalte P, Yeong ML, Holloway L et al. The Prognosis of adenocarcinomous carcinoma of the uterine cervix.Br. J. Obstet. Gynaecol 1992; 99:745-750.
22. Preeti, Kalhan S, Alka, jain K, Arora K S. Should Mucin Histochemistry Be Routinely Done For Carcinoma Cervix [12] World Health Organization (February 2006). "Fact sheet No.297: Cancer".
23. Benda JA, Platz CE, Buchsbaum H, Lifshitz S. Mucin production in defining mixed carcinoma of the uterine cervix: a clinicopathologic study. Int. J. Gynaecol . Pathol. 1985; 4:314-327.
24. Vatsala misra, SC Gupta, Anjali Goel, PA Singh: Re-classification of carcinoma cervix uteri by mucin histochemistry; Indian J. pathol microbiol; 1997 40(4); 463-468.
25. JV Bulmer, N.R. Griffin, RE-Kingston, M Wells. Minimal Deviation Adenocarcinoma (adenoma malignum) of the endocervix; A histochemical and immunohistochemical study of 2 cases (Nov 1988) Gynecol Oncol. Jan.1990 36(1); 139–146.
26. Vatsala misra, SC Gupta, Anjali Goel, PA Singh: Re-classification of carcinoma cervix uteri by mucin histochemistry; Indian J. pathol microbiol; 1997 40(4); 463-468.
27. Hayashi I, Tsuda H,Shimuda T.Reappraisal of orthodox histochemistry for the diagnosis of minimal deviation adenocarcinoma of cervix. J Surgical Pathol 2008 , 24:559-562.
28. SK Mathur, Nisha Marwaha, Ritu Arora Sumiti Gupta, Veena Gupta, B Arora. Significance of mucin secretion in carcinoma of uterine cervix. Indian J.pathol. microbial. July 2002 45(3); 261-264.
29. JV Bulmer, N.R. Griffin, RE-Kingston, M Wells. Minimal Deviation Adenocarcinoma (adenoma malignum) of the endocervix; A histochemical and immunohistochemical study of 2 cases (Nov 1988) Gynecol Oncol. Jan.1990 36(1); 139–146.