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A Comparative Study between the Histochemical Characteristics of Mucosubstances in Normal and Adenocarcinoma of Endocervical Glands

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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. Mucus plays an important role in reproductive function and defense of the female reproductive tract. Objectives: To know the mucin distribution of the endocervical glands in normal and malignant lesions. Methodology: This is a retrospective, observational, analytical, case control study aimed to evaluate mucins histo-chemical pattern in normal and malignant endocervical glands. Twenty five histologically proven blocks of normal and 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 normal endocervical glands; the epithelium and glands show mixture of both neutral and acidic mucins. Acidic mucins are more in amount than neutral. In acidic mucins, sialomucins are predominantly seen. In adenocarcinoma of endocervical glands; mixtures of both neutral and acidic mucins are found. Neutral mucins are in trace amounts. In acidic no sialomucins but few sulphomucins are seen. Conclusion: Mucin histochemical patterns serve as valuable, cost-effective tool for diagnosis in 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, Normal, Adenoarcinoma

1. 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.

2. 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.The type of study was observational, analytical and case control study. Sample size was 25 blocks of histologically proven normal and 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 used to prove their presence.
4) Alcian blue -- This stain can be used at various pH levels.
   a) AB pH 1.0 -- 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]

3. Observations and Results

During the period from May 2010 to June 2012, 25 blocks of normal and carcinoma endocervical glands were collected. Normal endocervical gland specimens were used as controls with adenocarcinoma as test. Histological technique was preceded and staining with H and E along with special stains such as PAS, PAS-Diastase, AB-pH 2.5 and 1.0, 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 normal and malignant endocervical glands

Sr. No.	Stains used	Inference about mucosubstances	
		Normal.	Adenocarcinoma endocervix.
1	H & E	Long, tubular, endocervical glands with coiling and branching at places and are lined by simple columnar epithelium exhibiting folds and some secretory cells.	Adenocarcinoma identified and confirmed.
2	PAS	PAS +ve substances present. Photomicrograph 1 (PAS 10X)	PAS +ve substances present. Photomicrograph 1a (PAS 10X)
3	PAS-Diastase.	No glycogen. Photomicrograph 2 (PAS-D 10X)	No glycogen. Photomicrograph 2a (PAS-D 10X)
4	PAS- PH	No neutral mucins. Photomicrograph 3 (PAS-PH 10X)	Trace neutral mucins. Photomicrograph 3a (PAS-PH 10X)
5	AB-pH 2.5	Acidic mucins present. Photomicrograph 4 (AB PH 2.5 10x)	Trace amount of acidic mucins. Photomicrograph 4a (AB PH 2.5 10x)
6	AB – pH1	Very few sulphomucins. Photomicrograph 7 (AB 1 -10X)	No Sulphomucins. Photomicrograph 7a (AB 1 -10X)

7	AB- PAS	Mixture of both acidic and neutral mucins. Photomicrograph 6 (AB-PAS 10X)	Trace amount of acidic mucosubstances. Photomicrograph 6a (AB-PAS 10X)
8	AF	Presence of few sulphomucins. Photomicrograph 5 (AF 10X)	Trace or no amount of sulphomucins. Photomicrograph 5a (AF 10X)
9	AF-AB	Mixture of both sulpho and sialomucins. Sialomucins are predominant. Photomicrograph 8 (AF-AB 10X)	Trace amount of sulphomucins. Photomicrograph 8a (AF-AB 10X)

Inference

Regarding comparison of mucin histochemistry of normal and malignant endocervix, a mixture of mucosubstances were found in both, those were neutral and acidic mucosubstances. In acidic mucosubstances reversal of mucin pattern was observed. In the present study, acidic mucins are more in amount than neutral in normal endocervix, whereas neutral mucins are in trace amounts. In acidic, no sialomucins but few sulphomucins are seen in adenocarcinoma endocervix.[15]

4. Discussion

Worldwide, cervical cancer is the fifth most deadly cancer in women.[16] It affects about 16 per 100, 000 women per year.[17] It is most important and accounts for 20-25% of all the cancers and 85% of all the female genital tract malignancies. Primary adenocarcinoma makes 5-10% of all cancers of the cervix. [18] The amount of stainable mucin may show marked variation in malignant lesion as compared to normal endocervical glands.[19] 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]

The term “mucosubstances” is used to denote all tissue components, other than glycogen, rich in carbohydrates which are present in connective tissue or as secretion of certain epithelial structures by Spicer et al.[6, 24]

Numerous types of mucins occur depending on the site of production. Example of connective tissue mucins are chondroitin sulphate, heparin sulphate, keratin sulphate and hyaluronic acid. Epithelial mucins may be neutral or acidic. Neutral mucins are hexosamine units which may be associated with glucoronic or sialic acid; the reactive group being carboxyl. In sulphated mucins this group is blocked by a sulphate group which becomes the active group[55]. 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-glucoronic acid). These can be enzymatically digested, though enzyme resistant forms do occur.[25]

The presence of carboxylated or sulphated groups was determined by various staining techniques and confirmed using enzyme digestion methods.[25]

5. Summary and Conclusion

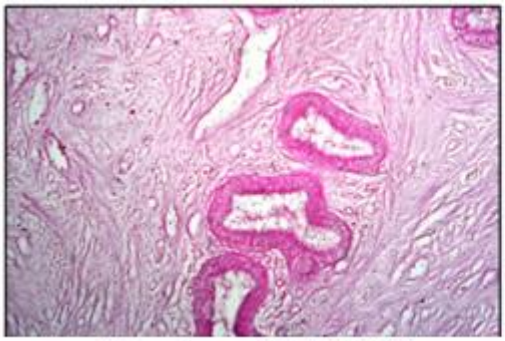
Mucin histochemistry of normal and malignant endocervical glands was undertaken in the department of Anatomy at Krishna Institute of Medical Sciences University, Karad from 2010 to 2012.

In the present study, routine and special histochemical methods were applied for comparison of mucins in normal and malignant endocervical glands.

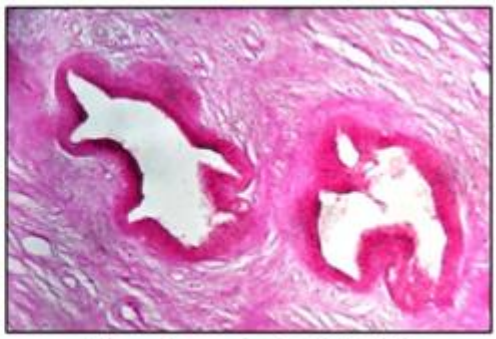
Haematoxylin and Eosin was used as routine stain for identification of tissue and confirmation of diagnosis of endocervical adenocarcinoma.

- 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.
- The varied heterogenecity of acidic mucins was indicated by a mixture of sulpho and sialomucins.
- In the present study mucin histochemistry of normal endocervical glands showed mixture of mucosubstances, both neutral and acidic. Acidic mucins were more in amount than neutral. In acid mucins, sialomucins were predominantly seen.
- 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.
- Reduction in amount or absence of neutral mucins in malignant lesion with corresponding increase in sulphomucin correlated well with various workers.
- In the present study no correlation between the age of the patient and mucin secretion could be observed in contrast to previous reports. This could be because of small sample size.
- Lesions diagnosed as moderately or poorly differentiated squamous cell carcinomas on H & E stain may turn out to be squamous cell carcinomas with mucin secretion, adenocarcinoma or adenosquamous carcinoma after staining with mucin stains depending upon the amount of mucin present. This emphasizes the importance of mucin stain as a routine tool for diagnosis of cervical carcinoma.
- Mucin content and type of mucin present can be regarded as an important prognostic indicator and early diagnosis may help in reducing the mortality related to endocervical malignancies.

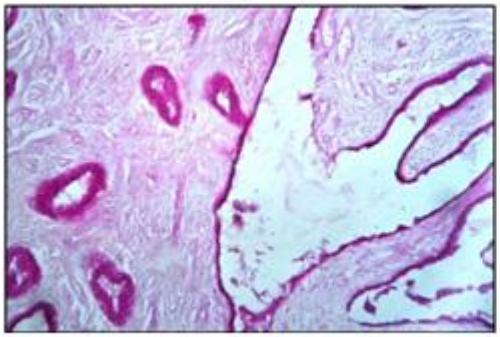
Normal Endocervical Glands



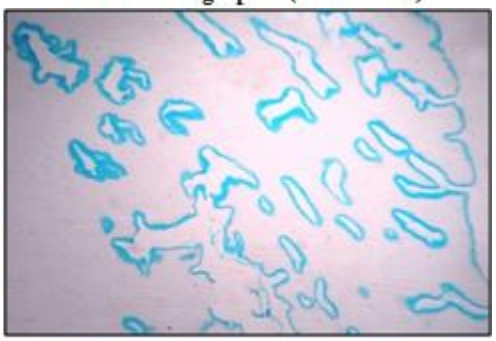
Photomicrograph 1 (PAS 10X)



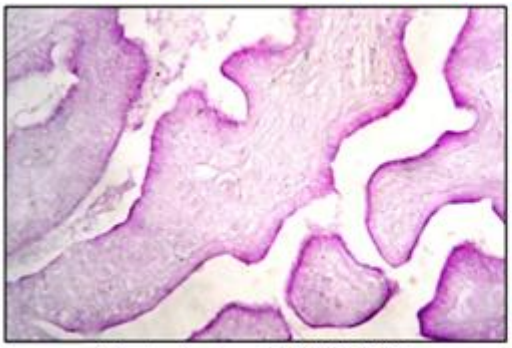
Photomicrograph 2 (PAS-D 10X)



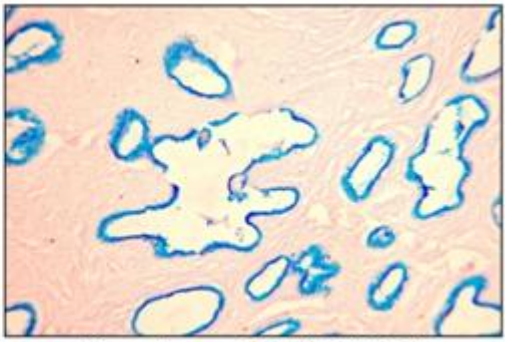
Photomicrograph 3 (PAS-PH)0))10X)



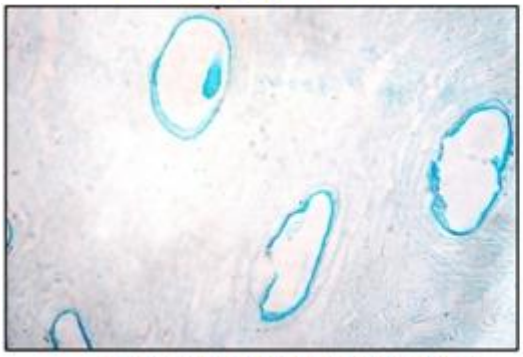
Photomicrograph 4 (AB PH 2.5 10X)



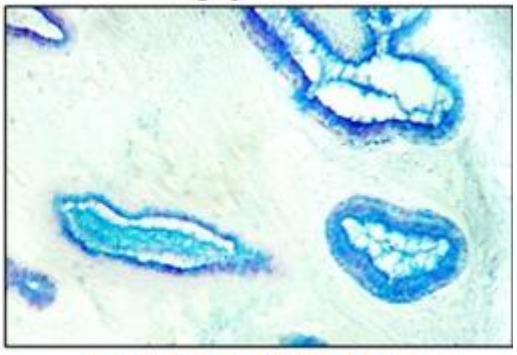
Photomicrograph 5 (AF 10X)



Photomicrograph 6 (AB-PAS 10X)

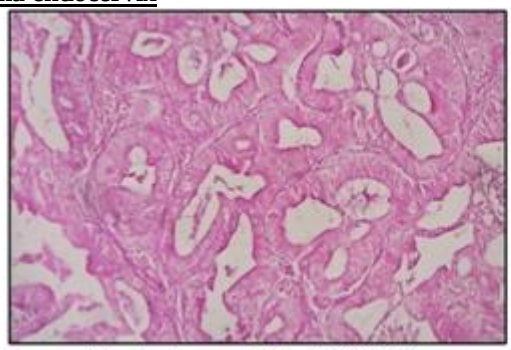


Photomicrograph 7 (AB PH 1, 10X)

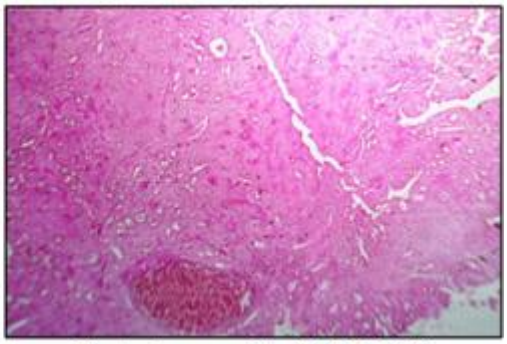


Photomicrograph 8 (AF-AB 10X)

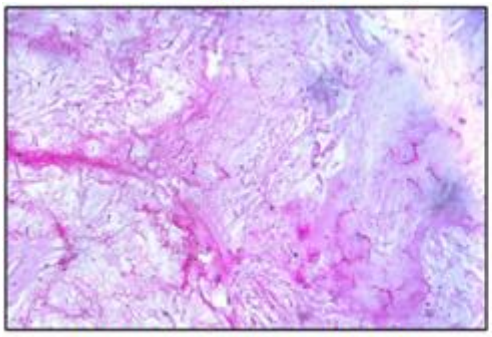
Adenocarcinoma endocervix



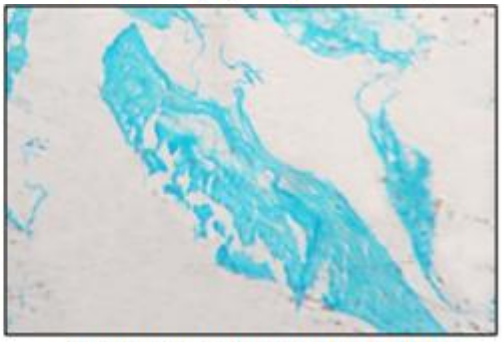
Photomicrograph 1a (PAS 10X)



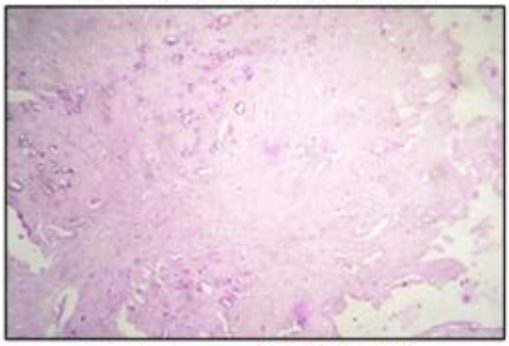
Photomicrograph 2a (PAS-D 10X)



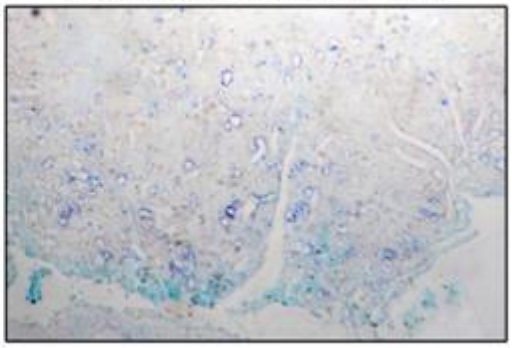
Photomicrograph 3a (PAS-PH 10X)



Photomicrograph 4a (AB PH 2.5 10X)

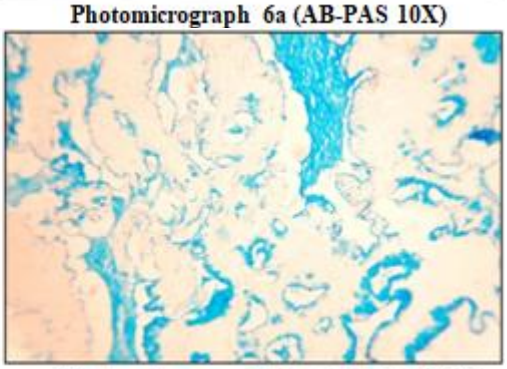


Photomicrograph 5a (AF -10X)



Photomicrograph 6a (AB-PAS 10X)

Photomicrograph 7a (AB 1 -10X)



Photomicrograph 8a (AF-AB 10X)

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