A Study of Histochemical Characteristics of Mucosubstances in Normal 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 defence of the female reproductive tract. **Objectives:** To know the mucin distribution in normal endocervical glands. **Methodology:** This is a retrospective, observational , analytical, case control study aimed to evaluate mucin histochemical pattern in normal endocervical glands. Ten histologically proven blocks of normal endocervical glands were taken. Tissue sections were stained by, PAS, PAS-diastase, Phenylhydrazine-PAS, Alcian blue pH 2.5, Alcian Blue pH 1, combined Alcian blue-PAS, Aldehyde fuschin and combined Aldehyde fuchsin-Alcian blue techniques. **Results:** Results were tabulated according to colour 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. **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,*

Introduction

Mucus is a complex viscous adherent secretion synthesized by specialized goblet cells in the columnar epithelium that lines all the organs that are exposed to the external environment. This includes the respiratory tract, the gastrointestinal tract, the reproductive tract and the oculo-rhino-otolaryngeal tracts.[1,2]Mucus plays an important role in reproductive function and defence 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]

Mucins are high molecular weight glycoproteins that are found dispersed throughout the epithelia of the gastrointestinal, respiratory and reproductive tract.[5] 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.[6] Connective tissue mucosubstances are called "mucopolysaccharides", while those secreted by epithelia are referred to as "mucins".[7]

Mucins perform a wide variety of functions like lubrication, protection against acids etc. The

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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.[8]

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.[9, 10, 11] weakly acidic mucins contain terminal carboxyl groups and are called as carboxylatedmucins 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.[10, 11]

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.[12]

Material and Method

The present study was conducted in the Department of Anatomy, Krishna Institute of Medical Sciences, University, Karad from May2010 to June 2012.The type of study was observational, analytical and case control study. Sample size was 10 blocks of histologically proven normal endocervical glands. Staining with 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.

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 colour intensity into

different grades ranging from + to ++++. ,[13, 14, 15]

Observations and Results

During the period from May 2010 to June 2012, ten blocks of histologically proven normal endocervical glands were collected. Histological technique with special stains as PAS, PAS-D, PAS-PH, AB 2.5 and 1, AF, combined AB-PAS, AF-AB was carried out.

All the results were tabulated according to colour intensity into different grades ranging from + to ++++.[13, 14, 15]

Colour Index:

++++ : Very strong positive reaction.

+++ : Strong positive reaction.

++ : Moderate reaction.

+ : Weak reaction.

- : Negative reaction.

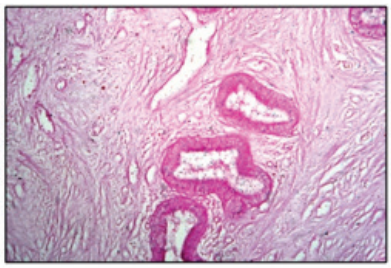
Table 1: Showing Histochemical result of normal endo-cervical glands.

Sr. No.	Stains used.	Result	Inference about Mucosubstances
1	PAS	+++	PAS +ve substances like carbohydrate & mucins present.
2	PAS- Diastase	+++	No glycogen, mucosubstances present.
3	PAS- Phenylhydrazine	++	Few neutral mucins present.
4	AB pH 2.5	+++	Acidic mucosubstances present.
5	AB pH 1	+	Very few sulphomucins present.
6	AB-PAS	++ blue + magenta	Mixture of both Acidic and neutral mucins. Acidic predominant.
7	Aldehyde Fuchsin	+	Presence of sulphomucins.
8	Combined Aldehyde Fuchsin- Alcian Blue	+++ Blue ++Purple	Mixture of both sulpho & sialomucins . Sialomucins are predominant.

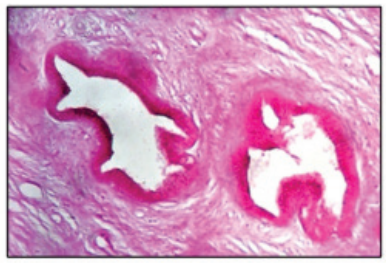
Inference:

Regarding mucinhistochemistry of normal endocervical glands, a mixture of mucosubstances was observed. The epithelium and glands are showing mixture of both neutral and acidic mucins. Acidic mucins are more in amount than neutral. In acidic mucins, sialomucins are predominantly seen.

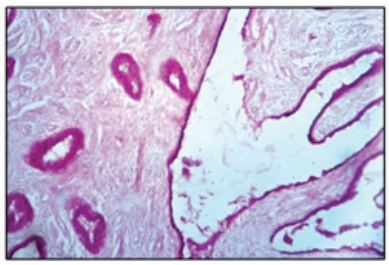
Normal Endocervical Glands



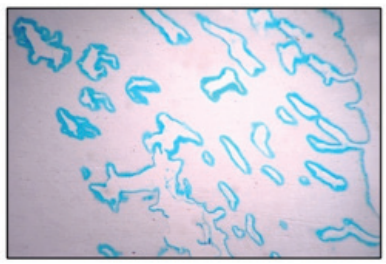
Photomicrograph 1 (PAS 10X)



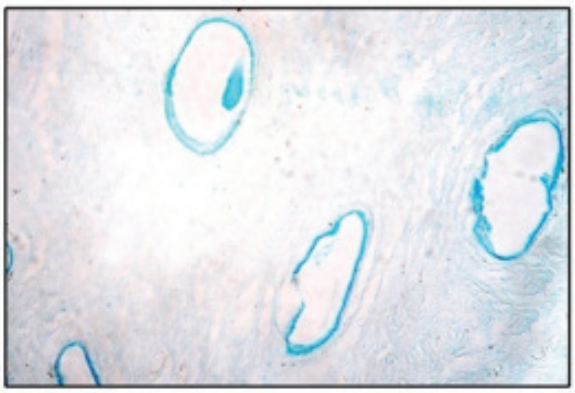
Photomicrograph 2 (PAS-D 10X)



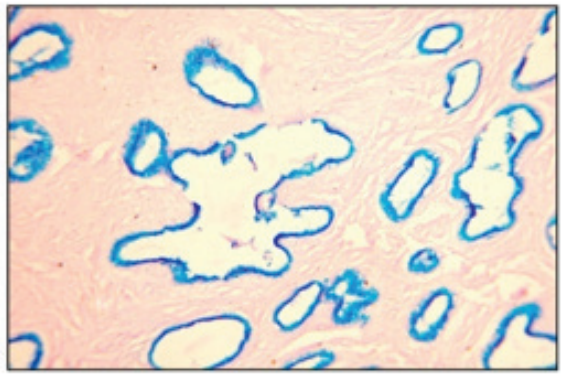
Photomicrograph 3 (PAS-PH)0))10X)



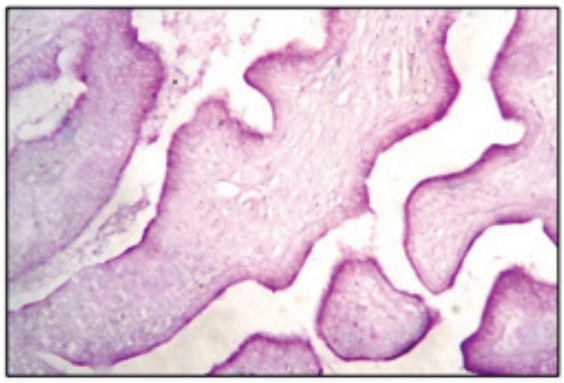
Photomicrograph 4 (AB PH 2.5 10X)



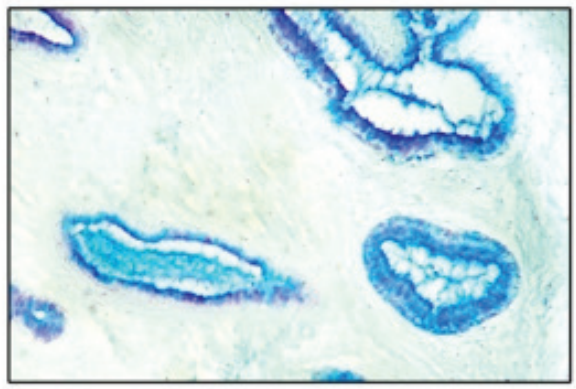
Photomicrograph 5 (AB PH 1 10X)



Photomicrograph 6 (AB-PAS 10X)



Photomicrograph 7 (AF 10X)



Photomicrograph 8 (AF-AB 10X)

Discussion

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, 15]

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[16]. 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.[16]

The presence of carboxylated or sulphated groups was determined by various staining techniques and confirmed using enzyme digestion methods.[16]

With the development of new histochemical methods by special stains, specific chemical composition of mucosubstances is documented by various scientists. But there have been very few studies on human endocervical mucosubstances such as by J. N. Bulmer et al (1988), Vatsala Misra et al (1997), Zhao Shumei et al (2003) and Hayashi, Isamu M.T et al (2003).

Table 2: Showing comparative study of mucin histochemistry of normal endocervix

Sr. No.	Worker	Inference/Observation.
1	Lapertosa G et al17(1986)	Abundant amounts of neutral mucins. Sialomucins were trace and predominant over sulphomucins.
2	Vatsala Misra et al 18(1997)	Mixture of neutral and acidic mucins, with neutral mucins being predominant.
3	Zhao Shumei et al19(2003)	Normal endocervical glands predominantly produce sulphomucins.
4	Present study	Mixture of neutral and acidic mucins. Acidic mucins are more in amount than neutral. Sialomucins are predominantly seen.

Summary and Conclusion

Mucin histochemistry of normal 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, special histochemical methods were applied.

· 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.

· In the present study the results were compared with various other studies and the results correlated with them.

· The varied heterogenecity of acidic mucins was indicated by a mixture of sulpho and sialomucins.

· In the present study mucinhistochemistry of normal endocervical glands showed mixture of mucosubstances, both neutral and acidic. Acidic mucins were more in amount than neutral. In acid mucinssialomucins were predominantly seen.

The present study correlates with workers like, Lapertosa G et al[17] V. Misra et al[18] Zhao and Shumei et al[19] in the histochemical study of normal endocervix

Ethical Clearance: Taken from institutional ethics committee, KIMSDU, Karad.

Source of Funding: KIMSDU.

Conflict of Interest: None

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