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Mucin histochemistry of normal and malignant endocervical glands

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Abstract

Aim: To assess mucin histochemistry of normal and malignant endocervical glands.

Material and method: 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, ABPAS, AB-PH2.5 and 1, AF and AF-AB was carried out and results were interpreted. Normal endocervical specimens were used as controls with carcinoma of endocervix as test. 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.

Result: The carboxylated/sialomucins were few as blue colour appeared as weak reaction. Mixture of neutral and acidic (sulphated) mucins was observed with AB-PAS staining.

Conclusion: This emphasizes the importance of mucin stain as a routine for diagnostic 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 regarding endocervical malignancies.

Keywords: Malignant endocervical glands, mucin histochemistry

Introduction

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

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^[5,6]. 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. 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 fuchsin-Alcian blue were used to simultaneously assess the proportions of various mucin types in the epithelium of endocervical glands examined.

With all the above view, present study has been undertaken to find out any variation present in mucosubstances in normal and malignant lesions of the cervix. In the present study comparison has been done between cervical glands of normal adult, menopausal women and women suffering from different grades of histologically proved CaCx^[7]. Only human adult

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menopausal women were considered because in the child bearing age the nature of cervical mucus varies in different phases of menstrual cycle. In proliferative phase it is thick viscus, while it changes to watery, thin, slimy fluid in secretory phase.

Material and method

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 10 blocks each of histologically proven normal and malignant endocervical glands. Work protocol was submitted to the protocol and ethical committee for approval and necessary permission was taken. The study was undertaken on 10 specimens each of normal and 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, ABPAS, AB-PH2.5 and 1, AF and AF-AB was carried out and results were interpreted. Normal endocervical specimens were used as controls with carcinoma of endocervix as test.

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.

The PAS technique is based upon the reactivity of free aldehyde groups within the monosaccharide units with the Schiff reagent to form a bright red magenta end product. The initial reaction in the PAS technique is the oxidation of hydroxyl (OH) groups attached to adjacent carbon atoms. These groups are referred to as 1, 2 glycols. Oxidation of these glycols results in the formation of Schiff reactive aldehyde groups. In most protocols, a 0.5% to 1.0% solution of periodic acid is used as the oxidant. The intensity of the color that develops following treatment with the Schiff re-agent is proportional to the concentration of reactive 1-2 glycol groups within the tissue. The PAS technique is particularly sensitive for the detection of neutral mucins as well as acid mucins that contain significant quantities of sialic acid. In addition to mucins, the PAS technique is also widely used for the detection of glycogen and various glycoproteins. The PAS technique is particularly valuable for the visualization of basement membranes due to the presence of Schiff reactive glycoproteins within these structures.³¹ The PAS reaction is useful indicator of the presence of tissue carbohydrates, and particularly so for glycogen when the technique incorporates a diastase digestion stage^[7].

Result

During the period from May 2010 to June 2012, ten blocks of histologically proven normal and malignant endocervical adenocarcinoma were collected. Normal endocervix specimen were used as control with malignant endocervical adenocarcinoma as test. Histological technique was preceded

and staining with H and E and with special stains as PAS, PAS-D, PAS-PH, AB 2.5 and 1, AF, combined AB-PAS, AF-AB was carried out. Hematoxylin and Eosin stain used for identification and confirmation of normal endocervix revealed glands lined by simple columnar epithelium. The glands are tubular, coiled and show branching. Regarding special stains, PAS stain was used to assess presence of neutral mucosubstances. PAS stain for normal endocervix gave magenta color with strong reactivity suggestive of presence of neutral mucin. Epithelium and glands, both gave moderate to strong reactivity. PAS-diastase stain gave magenta color in epithelium and glands suggestive of presence of PAS positive mucins and no glycogen. Normal endocervical glands in this gave moderate to strong positivity (Photomicrograph No.4). Diastase digestion followed by PAS was utilized to distinguish the diastase-resistant mucosubstances from glycogen.

Combined stains such as AB- PAS were carried out to assess neutral and acidic mucins. In AB-PAS stain mixture of colors was seen, with predominance of purple and magenta colour, suggestive of presence of sulphated mucins and neutral mucins respectively. The carboxylated/sialomucins were few as blue colour appeared as weak reaction. Mixture of neutral and acidic (sulphated) mucins was observed with AB-PAS staining. Further more, sulphated mucins were assessed by Aldehyde fuchsin stain. The Aldehyde fuchsin gave moderate to strong reaction as purple color. Combined stain AF-AB was done as it was a reliable means to separate sulphated from carboxylated mucins. Aldehyde fuchsin-alcian blue pH 2.5 gave strong reaction with purple colour and weak reaction as blue color suggestive of predominance of sialomucins with small amounts of sulphomucins in normal endocervix.

In the present study, H and E and special stains were used for mucin histochemistry of 10 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. Regarding special stains, PAS stain was used to assess 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

For acidic mucin, in all adenocarcinomas AB-pH 2.5 carried out gave mild reaction as blue colour. This is suggestive of presence trace amount 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. 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. 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. AF-AB technique helps to differentiate and confirm carboxylated and sulfated mucins. AF-AB on all 10 specimens of denocarcinoma cervix gave strong reaction as blue and weak reaction as purple colour suggestive of predominance of carboxylated mucins and traces of sulphated mucins.

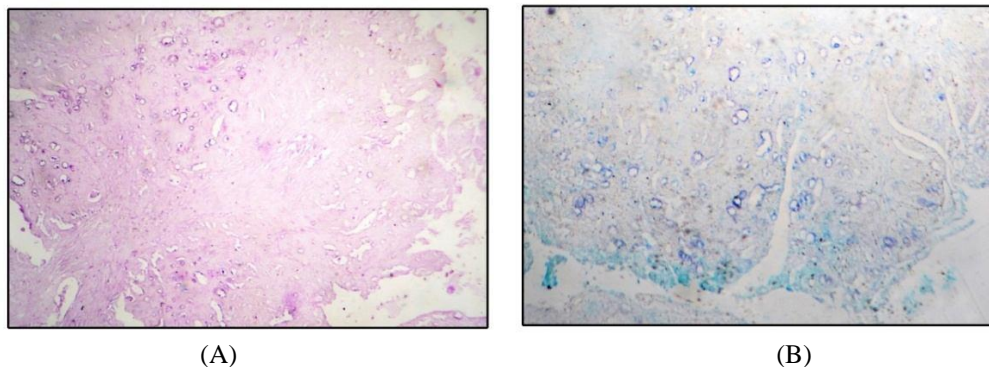


Fig 1: Adenocarcinoma endocervix A) Photomicrograph 15 (AB 1 10X); B) Photomicrograph 16 (AB-PAS 10X)

Discussion

Worldwide, cervical cancer is the fifth most deadly cancer in women.⁴⁵ It affects about 16 per 100,000 women per year.⁴⁶ 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.⁴⁷ The amount of stainable mucin may show marked variation in malignant lesion as compared to normal endocervical glands. Benda *et al.* were the first to demonstrate the importance of mucin secretion in cervical cancer which was confirmed later on ^[8]. 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 ^[9].

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.* ^[10] 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 glucuronic or sialic acid; the reactive group being carboxyl. In sulphated mucins this group is blocked by a sulphate group which becomes the active group⁵⁵. 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-glucuronic acid). These can be enzymatically digested, though enzyme resistant forms do occur ^[10]. The presence of carboxylated or sulphated groups was determined by various staining techniques and confirmed using enzyme digestion methods ^[10].

Lapertosa G, Baracchini P *et al.* studied 20 specimens of normal endocervix and 10 of adenocarcinomas. Normal endocervical glands showed abundant amounts of neutral mucins. Generally sialomucins were scarce and predominant over sulphomucins ^[11].

Vatsala Misra, *et al.* re-classified carcinoma cervix uteri by mucin histochemistry with 6 cases of adenocarcinomas and 12 normal. Sulfomucin was found to be predominant. Normal endocervical glands had a mixture of neutral and acidic mucins with neutral mucin being predominant.⁵⁸

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 ^[12].

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 ^[13].

Conclusion

The present study correlates with workers like, Zhao Shumei *et al.*, V. Misra *et al.* and Lapertosa G *et al.* in the histochemical study of normal endocervix and with Lapertosa G *et al.*, JV Bulmer *et al.*, V. Misra *et al.* and Hayashi *et al.* in the histochemical study of malignant endocervix. 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 for diagnostic 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 regarding endocervical malignancies.

Conflict of interest: No conflict of interest

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