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Pathology Section

Mucin Histochemistry of Endocervix in Health and Disease

DIVYA LAKSHMI[1], KUMARI JYOTHI[2]

ABSTRACT

Introduction: Mucus plays an important role in reproductive function and defence of the female reproductive tract. Alterations in mucus quantity and quality are related to physiological and pathological changes of female reproductive tract.

Aim: To study the specific types of endocervical mucin using standard histochemical stains in, proliferative phase of the menstrual cycle, secretory phase of the menstrual cycle, non-neoplastic lesions of the cervix and neoplastic lesions of the cervix.

Materials and Methods: Prospective study was conducted over a period of two years from September 2012 to August 2014. All cervical biopsies as well as cervices from hysterectomy specimens received at Department of Pathology at MVJMC and RH was taken for the study. Corresponding endometrial bits were also taken to assess the menstrual cycle phasing. Thirty cases from the proliferative phase and 30 cases from the secretory phase of menstrual cycle. Thirty cases from non-neoplastic cervical lesions and 15 cases from neoplastic cervical lesions. On each cervical biopsy six sections were cut and mucin histochemistry was carried out. Mucin staining was graded as +, ++, +++. Data was entered in Microsoft excel 2010 and statistics was analysed using SPSS version 16.0. The p-value was obtained by chi-square test.

Results: Acid mucin was predominant in proliferative phase; neutral mucin was predominant in secretory phase. In inflammatory lesions mucin produced was variable. Among the neoplastic cases one squamous cell carcinoma showed mucin secretion which was reclassified as adenosquamous carcinoma of cervix. Among the acid mucins produced, it was found that carboxylated and weakly sulphated mucin was secreted predominantly.

Conclusion: Histochemical study of endocervical mucins yielded different types of mucin in the proliferative and secretory phases. Carboxylated mucin was predominant in inflammatory states and sulphated mucin was a component of cervical polyp. Adenosquamous carcinoma can be detected more easily.

Keywords: Acidic mucin, Alcian blue, Menstrual cycle, Neutral mucin, Periodic acid schiff stain

INTRODUCTION

Endocervix secretes slippery mucus secretion at a rate of 20-60 mg/day. The consistency and viscosity of mucus secreted by endocervical glands varies depending on the phases of menstrual cycle. The mucus character changes from a thick viscous to a watery consistency in periovulatory period to allow sperm to penetrate into the uterus and thus helps in fertilization. Quantity and quality of mucus secreted by endocervical glands are related to various physiological and pathological conditions of the female reproductive tract [1,2].

Histochemical studies have showed that both acid mucins and neutral mucins were produced by the same cells, but that the quantity produced varied through the menstrual cycle. The amount of neutral mucin produced was found to be greater in the periovulatory period, while acid mucin showed variations in quantity throughout the menstrual cycle [3].

Mucin histochemistry helps in identifying the types of mucin secretion in various physiological and pathological conditions of the cervix. The periodic acid schiff reagent stains the neutral mucin; alcian blue stains the acidic mucin and alcian blue in different molarity stains the various types of mucin (carboxylated and sulphated mucin). The combined alcian blue/periodic acid schiff provides a broad means of detecting mucins [4,5].

The objective of this study was to evaluate the expression of mucin in various phases of the menstrual cycle, non-neoplastic and neoplastic lesions involving the uterine cervix and to determine whether a distinct phenotypic pattern exists in the various phases of menstrual cycle to differentiate between benign, premalignant, and malignant endocervical glandular lesions.

MATERIALS AND METHODS

The present study was a prospective study which was conducted in the Department of Pathology, MVJ Medical College and Research Institute, Bangalore, Karnataka, India, over a period of two years from September 2012 to August 2014, after due clearance from institutional Committee for Ethics with IEC no: MVJMCRH/IEC-01/2012.

Mucin in cervicies of healthy females was studied separately in proliferative phase and secretory phase.

Mucin in diseased females was classified under non neoplastic cervical lesions and neoplastic cervical lesions. One hundred and five cervical biopsies were obtained from both hysterectomy specimens and cervical punch biopsies. Corresponding endometrial bits were also taken to assess the menstrual cycle phasing. Thirty cases from the proliferative phase and 30 cases from the secretory phase of menstrual cycle. Thirty cases from non-neoplastic cervical lesions and 15 cases from neoplastic cervical lesions.

Consent was procured from all the 105 cases.

Inclusion Criteria: All cervical biopsies with adequate amount of tissue from the above mentioned condition.

Exclusion Criteria: Biopsies from pregnant women.

The specimens were fixed in 10% formalin. Representative bits were placed in cassettes, kept in fixative and processed in the automatic tissue processor for 16 hours. Paraffin tissue blocks were prepared and 3-4 µ thick sections were cut and stained with routine haematoxylin and eosin stain, Periodic acid-Schiff stain, Alcian blue at pH 2.5 and also at lower pH by changing the molarity 0.06 M, 0.3 M and 07 M.

0.06M - Carboxylated and weakly sulphated mucin.

0.3M- weakly and strongly sulphated mucin.

0.7M- strongly sulphated mucin.

Combined alcian blue and periodic acid Schiff (for both acid and neutral mucins)

High iron diamine stain was not included in the study as the stain was expensive.

-ve	Negative staining
±	± Weak or variable staining
+	Mild staining
++	Moderate staining
+++	Strong staining

According to visual estimation, the intensity of colour reactions of the histochemical methods was graded as follows [4,5]:

STATISTICAL ANALYSIS

Data was entered in Microsoft excel 2010 and statistics was analysed using SPSS version 16.0. The p-value was obtained by chi-square test.

RESULTS

The age of patients in the present study ranged from 25 to 70 years. The maximum number 55 cases (52.4%) were seen in the age group of 40-49 years and only one case was seen in the age group of >70 years (0.9%).

The cases ranged from Non-specific Cervicitis to Squamous Carcinoma. The incidence of Non-specific cervicitis was found to be highest in the present study 68 cases (64.8%), among non-specific cervicitis, endocervical glandular hyperplasia was noted in 28 cases (26.6%) and nabothian cysts was seen in 38 cases (36.1%).

In proliferative phase of menstrual cycle all the 30 cases had features of chronic non-specific cervicitis and mucin histochemistry showed combination of acid mucin as well as neutral mucins but the acid mucin was found to be the dominant component in the proliferative phase of menstrual cycle. Intensity of mucin staining in early proliferative phase was mild (+) and in late proliferative phase was severe (+++). The chi-square test: 2.99, p-value: 0.225 and the test were found not to be significant [Table/Fig-1].

Intensity Total 30 cases	PAS (Neutral mucin)	AB (Alcian blue)	p-value	Chi-square
Mild (+)	2	2	0.225	2.99
Moderate (++)	23	27		
Severe (+++)	5	1		
Total 30 cases	30	30		

[Table/Fig-1]: Mucinhistochemistry in proliferative phase.

Acid mucin at different molarity in proliferative phase showed varying degrees but the carboxylated and weakly sulphated mucin was found to be the dominant type. The p-value-0.0001, Chi-square test: 83.2; therefore, the test was significant [Table/Fig-2].

	Alcian Blue				
	0.06 M	0.3 M	0.7 M	p-value	Chi-square
Mild (+)	1	9	27	0.0001	83.2
Moderate (++)	6	19	3		
Severe (+++)	23	2	0		

[Table/Fig-2]: Acid mucin at different molarity in proliferative phase.

In secretory phase of menstrual cycle out of 30 cases studied, maximum number of cases i.e., 23 cases (76.7%) had features of chronic non-specific cervicitis followed by papillary endocervicitis six cases (20%) and one case of herpetic cervicitis.

Mucin histochemistry on cervix in secretory phase showed combination of both the mucins acid as well as neutral mucins but the neutral mucin was found to be the dominant component in the secretory phase of menstrual cycle. Intensity of acid mucin was more in late secretory phase as compared to early and mid-secretory phase. Intensity of neutral mucin remained constant as severe intensity (+++) in early mid and late secretory phase. The p-value-0.02, chi-square test 7.20 and the test was significant [Table/Fig-3].

Intensity Total 30 Cases	PAS	AB	p-value	Chi-square
Mild (+)	6	3	0.02	7.20
Moderate (++)	24	21		
Severe (+++)	0	6		
Total	30	30		

[Table/Fig-3]: Neutral and acid mucin in secretory phase.

Acid mucin at different molarity shows varying degrees but the carboxylated and weekly sulphated mucin was found to be dominant type. The p-value: 0.0001;chi-square: 93.6 and the test was significant [Table/Fig-4].

	Alcian Blue				
Intensity	0.06M	0.3M	0.7M	p-value	Chi-square
Mild (+)	0	10	29	0.0001	93.6
Moderate (++)	9	20	1		
Severe (+++)	21	0	0		
Total	30	30	30		

[Table/Fig-4]: Acid mucin at different molarity in Secretory phase.

Non-neoplastic conditions: Among the 30 non-neoplastic cervical lesions maximum number of cases (15 cases- 50%) had features of chronic non-specific cervicitis followed by papillary endocervicitis 8 cases (26.7%), 3 cases(10%) of herpetic cervicitis, 2 case (6.7%) of cervical polyp, 1 case (3.3%) of exophytic condyloma and follicular cervicitis. Mucin Histochemistry in non-neoplastic lesions (irrespective of menstrual cycle) showed combination of both acid and neutral mucin. The p-value: 0.022;chi-square test: 7.59 tests were significant [Table/Fig-5]. Acid mucin at different molarity in non-neoplastic lesion showed carboxylated and weakly sulphated mucin as dominant type and strongly sulphated mucin as minimal. In nabothian cysts and in endocervical polyps severe intensity of sulphated mucins was noted. The p-value: 0.0001; chi-square: 147 tests were significant [Table/Fig-6].

Intensity (Total 30 cases)	PAS	AB	p-value	Chi-square
Mild (+)	12	3	0.022	7.59
Moderate (++)	14	23		
Severe (+++)	4	4		
Total	30	30		

[Table/Fig-5]: Mucin histochemistry in non-neoplastic lesions of cervix irrespective of menstrual cycle.

Total 30 cases	Alcian Blue				
Intensity	0.06M	0.3M	0.7M	p-value	Chi-square
Mild (+)	1	1	29	0.0001	147
Moderate (++)	3	29	1		
Severe (+++)	26	0	0		
Total	30	30	30		

[Table/Fig-6]: Acid mucin at different molarity in non-neoplastic lesions.

Neoplastic lesions: Among the 15 neoplastic lesions Cervical Intraepithelial Neoplasia (CIN III) was seen in seven cases (46.7%), followed by Squamous cell carcinoma in six cases (40%) and two case (13.3%) of cervical fibroid. Mucin histochemistry in endocervical glands adjacent to leiomyoma was variable. In one

No.	H&E	Alcian Blue (in endocervical glands)	Alcian blue (in neoplastic cells)	PAS (in endocervical glands)	PAS (in neoplastic cells)	AB-PAS (in glands)	AB-PAS(in neoplastic cells)
1	Cervical fibroid	+++	NA	+++	NA	Equal	NA
2	Cervical fibroid	+	NA	+	NA	Equal	NA
3	CIN III	+++	-ve	+++	-ve	Equal	-ve
4	CIN III	+	-ve	+	-ve	Equal	-ve
5	CIN III	+	-ve	+	-ve	+	-ve
6	CIN III	+	-ve	+	-ve	Equal	-ve
7	CIN III	+	-ve	+	-ve	Equal	-ve
8	CIN III	+	-ve	+	-ve	AB>PAS	-ve
9	CIN III	+	-ve	+	-ve	Equal	-ve
10	Well diff SCC	*	-ve	*	-ve	*	-ve
11	Mod diff SCC**	+	+	+	+	Equal	+
12	Well diff SCC	*	-ve	*	-ve	*	-ve
13	Well diff SCC	*	-ve	*	-ve	*	-ve
14	Well diff SCC	*	-ve	*	-ve	*	-ve
15	Mod diff SCC	*	-ve	*	-ve	*	-ve

[Table/Fig-7]: Mucin histochemistry in neoplastic lesions – total 15 cases.
* Mucinhistochemistry on squamous cell carcinoma had no role; **One case of squamous cell carcinoma showed positivity for mucin staining; Equal here means glands showed positivity for both acid and neutral mucin with same intensity

cervical fibroid the endocervical glands adjacent to the neoplasm showed severe intensity of both acid and neutral mucin. Another cervical fibroid showed mild intensity of both acid and neutral mucin in the endocervical glands, probably due to the stretch effect of the leiomyoma which hindered mucin secretion. There was no significant variation in the mucin produced in the endocervical gland with regard to the type of neoplasm. One moderately differentiated squamous cell carcinoma was recategorized as Adenosquamous carcinoma after mucin histochemistry.

In Cervical Intraepithelial Neoplasia (CIN) III, no significant mucin production could be seen in the neoplastic epithelium [Table/Fig-7].

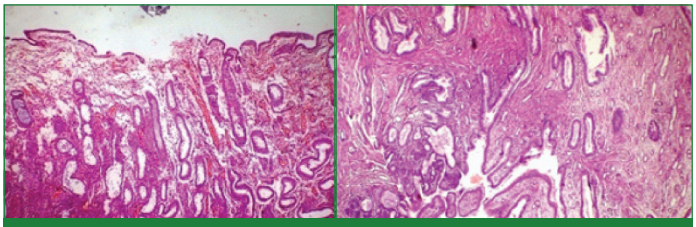
Connective tissue staining was mainly seen with alcian blue as mild intensity in non-neoplastic and moderate intensity in neoplastic lesions of cervix, showing the presence of hyaluronic which is a response to the adjacent tissue.

DISCUSSION

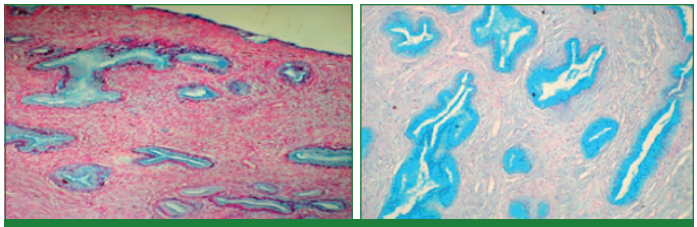
Cervical mucus secretions varies cyclically both in quality and quantity and this has been observed by various investigators [1,2,6]. Acid mucin and neutral mucin are produced by the same cells but the physiological and pathological conditions of endometrium like hormonal changes (oestrogen and progesterone phases) or infections can cause this variations in secretions [6].

Our study showed mucin secretion predominant in 4th and 5th decade of life and least in 7th and 8th decade of life since glandular changes, papillary endocervicitis, and nabothian cysts (38 cases-36%) was noted prominently in the reproductive age group.

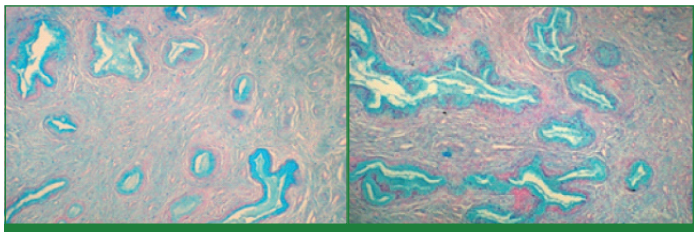
The present study involved 60 cases in menstrual cycle (mucin histochemistry in healthy females), [Table/Fig-8-15] demonstrates mucin histochemistry in cervicies corresponding to the endometrium in secretory phase healthy females. Thirty non-neoplastic cases and 15 cases of neoplasia (mucin histochemistry in diseased females). [Table/Fig-16-20] demonstrates mucin histochemistry diseased



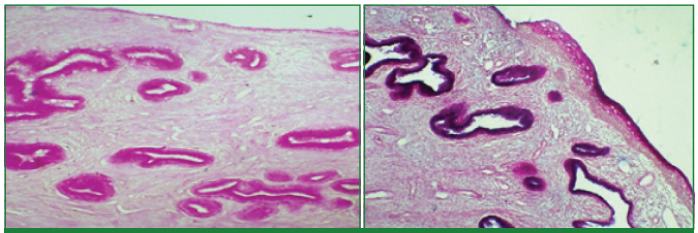
[Table/Fig-8]: Endometrium-secretory phase (x 40) H&E stain.
[Table/Fig-9]: Cervix-Chronic non-specific cervicitis (x 40) H&E stain.



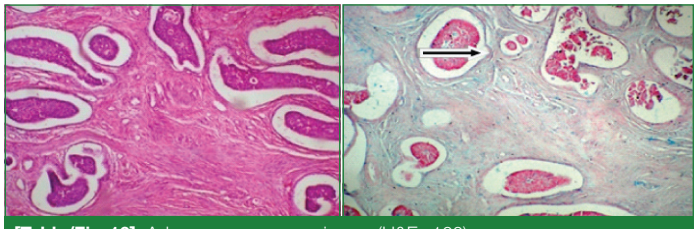
[Table/Fig-10]: Alcian Blue (x 100)-Acidic mucin (++)non-specific cervicitis.
[Table/Fig-11]: Alcian Blue 0.06 M (x 100)-Carboxylated mucin and weakly sulphated mucin (+++) non-specific cervicitis.



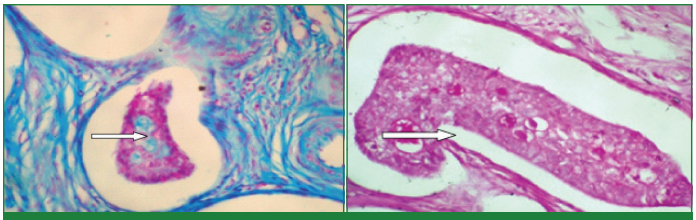
[Table/Fig-12]: Alcian Blue 0.3M (x 100)- Weakly and strongly sulphated mucin (++) non- specific cervicitis.
[Table/Fig-13]: Alcian Blue 0.7M (x 100)- Strongly sulphated mucin (+) non-specific cervicitis.



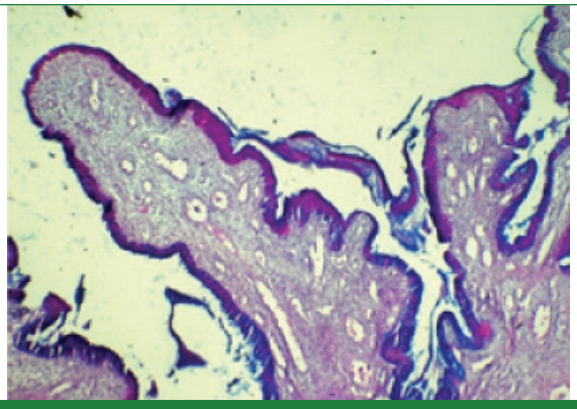
[Table/Fig-14]: Periodic acid schiff (x 100): Neutral mucin (++). (Non- specific cervicitis).
[Table/Fig-15]: Combined alcian blue and periodic acid schiff stain (x 100) – both acid and neutral mucins are present in equal intensity (purplish tinge).



[Table/Fig-16]: Adenosquamous carcinoma (H&E x100)
[Table/Fig-17]: Alcian Blue (x 100)-Acidic mucin (+) case of Adenosquamous carcinoma.



[Table/Fig-18]: Alcian Blue 0.06M (x 400)– Carboxylatedmucin, case of Adenosquamous carcinoma.
[Table/Fig-19]: Periodic acid schiff (x 400)-Neutral mucin (+)case of Adenosquamous carcinoma.



[Table/Fig-20]: Combined alcian blue and periodic acid schiff stain (x 100)–both acid and neutral mucin are present in equal intensity (purplish tinge). Case of cervical polyp.

females. Most studies have shown that both acid and neutral mucins are produced by the same cell, but the quality produced varied during the phases of menstrual cycle [3].

Proliferative Phase

In proliferative phase though the endocervical glands showed combination of acid mucin as well as neutral mucins the acid mucin component was dominant. Since the p-value was 0.225 and chi-square on this parameter was 2.99, test was found not to be significant. According to the study done by Wakefield EA et al., both acidic and neutral mucins are produced by cervical mucosal cells. The acidic mucin component undergoing cyclical changes are produced far in excess of neutral mucin [7]. These authors found an increase in sialoglycoproteins in the periovulatory phase concluding that sialoglycoproteins played a role in the production of sperm penetrable at mid cycle [7].

The present study also showed that different types of acid mucin are present in varying degrees but the carboxylated and weakly sulphated mucin is found to be the dominant type (76.6%). The p-value in the present study was 0.0001 and Chi-square was 83.2 therefore the test was significant. This correlated with the study of Wakefield EA et al., who concluded that sialoglycoprotein component of carboxylated mucin was found to be the dominant type [7]. According to Lapertosa G et al., sulphomucins is scarce or absent in proliferative phase [8], but present study, showed that these mucins are a component in the proliferative phase, of which strongly sulphated mucin was the predominant component in 60% of cases. On the contrary, Nirmala V et al., found neutral mucin to be greater in the periovulatory period. These authors also found variations in acid mucin production in the various phases of menstrual cycle [3].

Secretory Phase

Majority of endocervix showed combination of both the mucins acid as well as neutral mucins but the neutral mucin was found to be the dominant component in the secretory phase of menstrual cycle. The p-value was 0.02 and chi-square test was 7.20 indicating that mucin histochemistry in secretory phase was significant. While the present study reiterates neutral mucin increases in amount after ovulation. Nirmala V et al., have stated neutral mucin to be the greater component before as well as after ovulation [3].

The p-value was 0.0001 and chi-square was 93.6 tests, indicating that acid mucin on different molarity was significant with the major component being the carboxylated and weakly sulphated mucin.

According to the study done by Nirmala V et al., [3] the acid mucin was found to be made up of carboxylated and sulphated mucins; whereas, Wakefield EA et al., also found an increase in carboxylated mucin (sialomucins) in secretory phase and decrease in the sulphomucins [7]. In comparison though weakly sulphated mucin was seen in our study we did not identify secretory phase component of sulphomucins. These changes can be of great significance, since cervical mucus acts as a medium of support and transport of spermatozoa near the time of ovulation, but becomes increasingly nonreceptive to spermatozoa during the post ovulatory period.

In Non-Neoplastic Phase

Irrespective of menstrual cycle, all non-neoplastic cervical lesions also showed combination of both acid and neutral mucin. The p-value was 0.022 and chi-square was 7.59 indicating that mucin histochemistry in non-neoplastic lesions of cervix was not significant. According to Misra V et al., the inflammatory lesions contain neutral mucin predominantly which will be replaced by sialomucin and sulphomucin in endocervical polyps [9].

Typing of acid mucin showed that carboxylated and weakly sulphated mucin was the dominant type in non-neoplastic cervical lesions and strongly sulphated mucin was minimal. The p-value was 0.0001 and chi-square was 147 indicating that mucin histochemistry was significant in identifying the type of mucin in non-neoplastic lesions of cervix.

According to the study done by Lapertosa G et al., in chronic cervicitis a slight amount of sulphomucins is produced in the residual glandular epithelium, especially in areas presenting severe inflammation, pseudoerosion, or both was observed and the Nabothian cysts contained mainly sulphomucins [8].

In Neoplastic Lesions

Mucin histochemistry in endocervical glands adjacent to leiomyoma was variable. The type of acid mucin obtained in these glands reflected the same appearance as in the non-neoplastic lesions.

Among the seven CIN III cases, the glands adjacent to CIN showed variable intensity of both acid and neutral mucin but no mucin was found in the intraepithelial neoplastic cells. Among the sub type of acid mucin carboxylated and weakly sulphated mucin was found to be the predominant component. According to Wakefield EA et al., the glands adjacent to abnormal squamous epithelium and CIN, the acid mucin is variable with decrease in sulphomucin and increase in sialomucin (component of carboxylated mucin) [7,10].

In the SCC studied, the neoplastic cells had invaded the whole of endocervix with no normal endocervical glands. Among them one case showed mild intensity of both acid and neutral mucin within the neolumina of the neoplastic cell nests, rest of the neoplastic cases were negative for mucin in the neoplastic cells, after histochemistry diagnosis changed from SCC to adenosquamous carcinoma. There was no significant variation in the mucin produced in the endocervical gland with regard to the type of neoplasm. One moderately differentiated SCC was recategorised as Adenosquamous carcinoma after mucin histochemistry.

This has also been emphasised by Misra V et al., who have studied mucin histochemistry on SCC in order to pick up more cases of carcinoma with evidence of mucin secretion which can be missed on routine haematoxylin and eosin stains [7].

LIMITATION

As the present study is from a single institution the sample load on endocervical malignancies especially adenocarcinomas was not encountered in two years. Therefore, mucin histochemistry on neoplastic glandular epithelium must be studied and compared.

CONCLUSION

Mucin expressions differed among the various phases of menstrual cycle as well as in non-neoplastic and neoplastic cervical lesions. Histochemical study shows that Periodic acid schiff and Alcian blue are localising stains for neutral and acid mucins as a whole. Alcian blue stain at varying molarity identifies the subtype of acid mucins. Acid mucin was the predominant type of mucin in the proliferative phase and neutral mucin was the predominant type of mucin in the secretory phase. In inflammatory lesions mucin production was variable sulphomucin (acidic) being the predominant one and in glands adjacent to the neoplastic conditions carboxylated mucin was noted to be prominent. This conveys that acid mucin is prominent in pathological conditions of cervix.

Considering the limitations of the present study further research with cervical adenocarcinomas should be conducted.

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PARTICULARS OF CONTRIBUTORS:
1. Assistant Professor, Department of Pathology, The Oxford Medical College, Bengaluru, Karnataka, India.
2. Assistant Professor, Department of Pathology, KBNIMS, Gulbarga, Karnataka, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Kumari Jyothi,
98, Bhagyawanti Nagar, New Jewargi Road, Gulbarga-585102, Karnataka, India.
E-mail: jyothivibhute13@gmail.com

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