

Significance of Mucin Stains in The Diagnosis of Carcinoma of Cervix

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ABSTRACT

Background: As many as 25-35% of carcinoma cervix without clearly defined glandular structure on histopathological examination by Hematoxylin and Eosin stains have intracellular mucin demonstrable by using mucin stains which may redefine the classification of cervical carcinomas which becomes important as mucus secreting neoplasms resembling pure squamous cell carcinomas are more aggressive and have worse prognosis than their purely squamous counterparts.

Methods: Our study conducted at Assam Medical College Dibrugarh for a period of one year. 54 cases of carcinoma cervix diagnosed on Hematoxylin and Eosin stain were subjected to mucin stains i.e. Periodic acid Schiff and Alcian blue at pH 2.5 and the results were compared with each other.

Result: Out of the total 54 cases, 47 cases were squamous cell carcinoma (SCC, 87.03%) which were further graded as well differentiated type (12/47) SCC, moderately differentiated (31/47) SCC, poorly differentiated (4/47) SCC and only 3 cases were adenosquamous carcinoma (3/54; 5.5%) and adenocarcinoma (4/54; 7.41%) by conventional H&E stain. On subjecting to specific mucin stains, however 21.2% (10 cases) of the SCC (47 cases) contained intracellular mucin with varying percentage, along with adenocarcinoma and adenosquamous carcinomas. Revised diagnosis after application of mucins stains showed pure squamous cell carcinomas (37/54) i.e. 68.51%, adenocarcinomas remained same at 7.41% (4/54) and adenosquamous carcinoma increased (13/54) to 24.07%.

Conclusion: Identifying adenosquamous carcinomas after application of mucin stains which were not detectable by H&E stained sections has sparked academic interest because of its prognostic significance as it has been found in various studies that adenosquamous carcinomas often pursue an aggressive course and are associated with worse prognosis than their pure adeno- and squamous – counterparts as most of them are poorly differentiated. So recognition of this type of carcinomas can help to predict prognosis and to plan management.

Keywords: Carcinoma Cervix, Mucin, PAS & Alcian Blue

Introduction

Carcinoma cervix ranks the fourth most common cancer in women worldwide, and the seventh overall and its incidence varies with continents with greater percentage of the global onus found in the less developed regions, as the sensitivity of the screening tests in several low resource countries is rather poor so cervical carcinoma still continues to be the leading cause of cancer in females in developing countries and by the time the cases are detected, they tend to be at later stages of the disease.^[1, 2] Multifactorial causation, potential for prevention, and the sheer threat it poses make cervical cancer an important disease for in-depth studies, as has been attempted by this study.

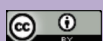
Squamous cell carcinomas comprise the majority of the cancers of the cervix accounting for 80% of all cervical cancers followed by adenocarcinoma which constitutes about 10-20% of cervical cancer cases. Adenosquamous and neuroendocrine carcinomas are rare cervical tumors. Routine application of stains for mucin, preferably

PAS and Combined alcian blue/PAS has shown that by classifying of carcinomas of cervix in H & E stained section alone, many mucin secreting neoplasms escape the detection unless mucin stain is applied and shown that 20 to 30 % of the cervical tumours regarded as being squamous cell carcinomas have to be reclassified either as poorly differentiated adenocarcinomas or as mixed adenocarcinomas. This reclassification is important and significance of identifying mixed tumours lies in the fact that these neoplasms run an unusually aggressive course and is associated with a much worse prognosis than their pure squamous and adeno- counterparts.^[3]

The primary objective of our study is to find out prevalence of mucin secretion in cervical carcinoma diagnosed on the basis of Hematoxylin & Eosin, (H&E) staining by application of mucin stains.

Materials and Methods

The present study was conducted from June 2015 to May 2016 in the Department of Pathology at Assam Medical



College, Dibrugarh during which total cases of 54 already diagnosed as cervical carcinomas on histopathological examination by Hematoxylin & Eosin staining were studied. A detailed clinical history, clinical examination and necessary investigations were obtained in each case and gross examination of each resected mass was done in a fastidious manner and representative samples were subjected to histopathological examination. The tissues were fixed by using 10% formalin and processed according to our laboratory standard operating procedure, stained with H&E and subsequently Periodic Acid Schiff's reagent (PAS) and Combined Alcian blue at pH 2.5/ PAS. Separate tissue controls were put for PAS and Alcian blue. The tumors were classified initially on H&E stained sections according to WHO classification [4]. After mucin stain they were re-classified into Fox classification system [5]. According to this, tumors with squamous growth pattern, keratin formation, intercellular bridges and no mucin positivity were classified into squamous cell carcinoma. The latter is subdivided into well, moderate and poorly differentiated. Tumors with acinar differentiation or widespread mucin secretion in atleast 75% of the tumor volume were labeled as adenocarcinoma. Lesions exhibiting both squamous and acinar differentiation with the minor component constituting at least one third of the tumor were considered as adenosquamous carcinoma. Squamous carcinoma exhibiting smaller quantities of mucin (not more than 30% of the tumor volume) were diagnosed as squamous cell carcinoma with mucin secretion.

Table 1: Showing Presenting Symptoms.

SYMPTOMS	NUMBER (n)	PERCENTAGE (%)
Abnormal Bleeding P/V	47	87.04
Discharge P/V	36	66.67
Pelvic pain	21	38.89
Post Coital Bleeding	14	25.93
Pain during Coitus	13	24.07
Others	11	20.37

Table 2: Showing Gross Findings in Per-Speculum/Colposcopic Examinations.

SPECULUM COLPOSCOPIC FINDING		NUMBER (n)	PERCENTAGE (%)
Cervical Ulcer		18	33.33
Cervical Growth	Exophytic Growth	21	38.8
	Infiltrative Growth	15	27.7
TOTAL		54	100.00

Table 3: Showing Revised Histopathological Diagnosis After Inclusion of Mucin Stains

DIAGNOSIS	Initial diagnosis on H&E		After mucin stains		P value
	N	%	n	%	
Squamous cell carcinoma	47	87.04	37	68.5	0.036
Adenocarcinoma	04	7.4	04	7.4	1.0
Adenosquamous carcinoma	03	5.5	13	24.07	0.013
TOTAL	54	100	54	100	

Result

From the total 54 cases, 47 were squamous cell carcinoma out of which 12 were graded well differentiated type, 31 were moderately differentiated and 4 as poorly differentiated, 3 were of adenosquamous carcinoma and 4 were adenocarcinoma. The mean age of presentation of carcinoma was 48.45 years with age groups ranging from 25 years-71 years. Most common presenting symptom was found to be abnormal vaginal bleeding/ postmenopausal bleeding (87.04% of cases) followed by discharge per vagina, pelvic pain, post coital bleeding etc as shown in Table 1. In present study most common per-speculum/colposcopic finding was found to be cervical growth and cervical ulceration. Out of the 47 cases diagnosed as squamous cell carcinoma, 10 cases i.e. 21.2% contained intracellular mucin with varying percentage, all the adenocarcinoma and adenosquamous carcinomas contained mucin. (Fig. 2- Fig. 4) After subjecting to mucin stains i.e. both PAS and Combined Alcian Blue at pH 2.5/PAS, only 68.5% turned out to belong to pure squamous cell carcinomas whereas the percentage of adenocarcinoma remained same and adenosquamous carcinoma rose to 24.07% respectively.

The statistical analysis using Chi square test with Yates correction in diagnosis of squamous cell carcinomas and adenosquamous carcinomas on H&E stains and after subjecting them to mucin stains showed statistically significant at $p < 0.05$ as shown in Table 3, however there was no statistical significance in the diagnosis of adenocarcinomas before and after doing mucin stains.

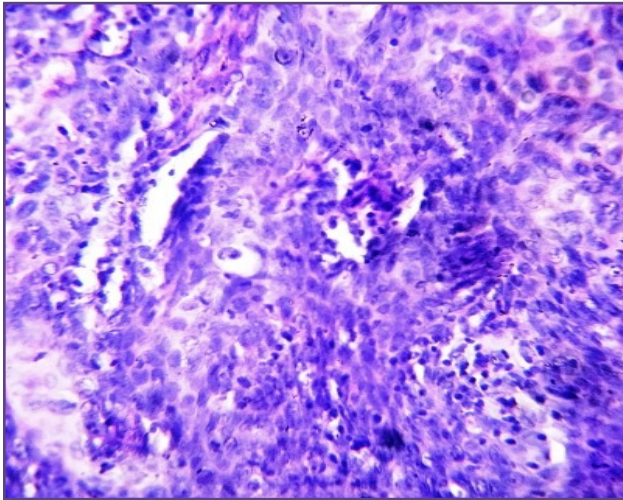


Fig. 1: Well Differentiated Squamous Cell Carcinoma with Negative PAS staining (40X).

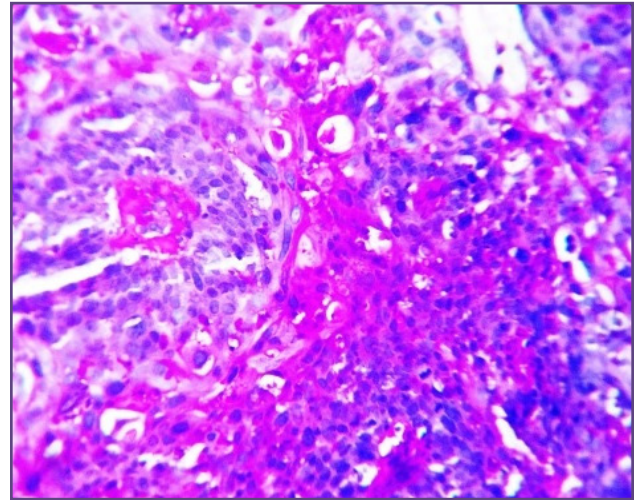


Fig. 2: Moderately differentiated SCC with PAS stained mucin in Tumor Cells.

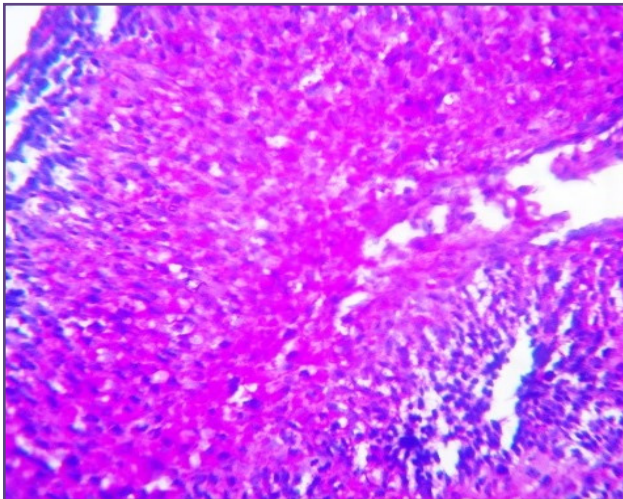


Fig. 3: Poorly Differentiated SCC with mucin content >80% of the tumor (PAS/40X).

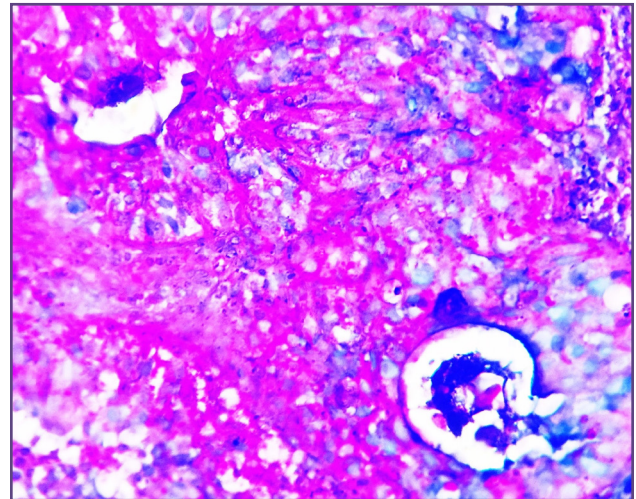


Fig. 4: Adenosquamous Carcinoma showing Combined Alcian Blue/PAS staining in the glandular component as well as other tumor cells(40X).

Discussion

Cervical cancer is a worldwide problem and ranks the fourth commonest cancer in women worldwide, and with statistics varying from country to country and from race to race. In the United States, the incidence rate has decreased during the last half century as a result of the widespread use of cervical cytologic screening programs but the same cannot be said of developing countries. Mortality due to cervical cancer is another indicator of health inequities as 86% of all deaths^[6] due to cervical cancer are in developing, low- and middle-income countries. Cervical cancer is mainly categorized into squamous cell carcinoma, adenocarcinoma and adeno-squamous 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.^[3] This gives much weight on the significance of mucin stains as a complementary to routine H&E staining for diagnosis of cervical carcinoma. In our study, we used two well established techniques, PAS and Combined Alcian Blue/PAS, for the detection of mucins in cervical carcinomas. Revised diagnosis after application of mucins stains showed pure squamous cell carcinomas to be 68.51%, adenocarcinomas remained same at 7.41% and adenosquamous carcinoma rose to 24.07% i.e. increased to 13 cases from 3 cases.

Our study revealed that combined AB/PAS stain preferable due to greater specificity and intensity of staining. Even though PAS also gives good results it has its drawback that, keratin also take the same stain and increases the chances of false positivity, but careful examination showed that the positivity shown by keratin was granular, it is different from block shown by mucin. The combined Alcian blue/PAS technique is a simple procedure and appears to differentiate sharply between acid mucins and neutral mucins.

Misra V et al. [7] studied biopsies of cervix uteri histochemically by using mucin stains, PAS, combined Alcian Blue/PAS, seventeen percent cases of squamous cell carcinomas showed positivity in mucin staining. Studies conducted by *Preeti et al.* [3], *Mathur SK et al.* [8], *Keshav P et al.* [9] also had similar findings. Observing most of the studies that have been done, it can be inferred that squamous cell carcinomas needs special mention as regards to mucin stain as it produce significant change in its diagnosis after application of mucin stains and diagnosis of most of the adenocarcinomas and the adenosquamous carcinomas remain unchanged before and after application of mucins. However, a much larger study over a longer period of time would accurately ascertain the worth and utility of routine mucin histochemistry as complementary to histopathological diagnosis of cervical carcinomas giving special emphasis on the poorly differentiated and moderately differentiated squamous cell carcinomas.

Conclusion

The role of histopathology as the benchmark for diagnosing invasive cervical carcinomas is undebatable and it still remains today the basic cornerstone of morphological identification of invasive cervical carcinomas but to advocate incorporation of mucin stains in the routine histological work up for diagnosis of cervical carcinomas would not be an understatement, especially poorly differentiated ones as it is of significance in determining the precise histopathological diagnosis.

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