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ORIGINAL ARTICLE

Should Mucin Histochemistry Be Routinely Done For Carcinoma Cervix

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ABSTRACT

Aim: The primary objective of our study is to find out prevalence of mucin secretion in cervical carcinoma by mucin histochemistry with special reference to squamous cell carcinoma; diagnosed by Haematoxylin and eosin (H&E) stain and to find the relative incidence of invasive carcinoma on the basis of H&E stain versus mucin stains.

Materials & Methods: Biopsies from 223 cases of carcinoma cervix were subjected to H&E stain and mucin stains i.e. Periodic acid schiff with diastase (DPAS) and Alcian blue at pH 2.5. Interpretation of cases on the basis of H&E staining alone and on the basis of mucin stains was done and the results were compared with each other.

Results: Morphological assessment on the basis of H&E stain showed 201(90.1%) cases of squamous cell carcinoma, out of these 132(59.2%) cases were moderately differentiated and 62(27.8%) cases were poorly differentiated. On application of mucin stains 29(13.0%) cases and 2(0.9%) cases out of 201 cases of squamous cell carcinoma were re-classified as squamous carcinoma with mucin secretion and adenosquamous carcinoma respectively.

Conclusion: Mucin stains should be done routinely on moderately and poorly differentiated squamous cell carcinoma for evidence of mucin secretion which can be missed on H&E stain. Such carcinomas are known to have a more aggressive clinical course associated with a poorer survival when compared to non-mucin secreting squamous cell carcinoma.

Key words: Mucin, cervix and carcinoma.

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Introduction

Carcinoma of the uterine cervix is an important malignancy and accounts for 20 - 25% of all the cancers in women. Approximately 80% of cervical cancer occurs in developing countries [1]. Carcinoma cervix has been the important cancer in women in India over the past two decades. Based on data generated by Population Based Cancer Registries (PBCRs) under ICMR in year 2009, changing trends have been seen in the incidence of carcinoma cervix. Since over 70% of the population in India resides in the rural areas, cancer cervix still constitutes the number one cancer in the rural population [2]. Squamous cell carcinoma constitutes the majority of all cervical cancers about 70% and primary adenocarcinoma make 10 -20 % [3].

Approximately 25-35% of carcinoma lacking definitive glandular structure have intracellular mucin demonstrable with the use of mucin stains [3],[8] .This lesion has been named as squamous cell carcinoma with mucin secretion or mixed carcinomas as classified by Fox system of classification [9].So, routine application of mucin stains have shown that 20 -30% of cervical carcinomas regarded as squamous cell carcinomas have to be re-classified into squamous cell carcinoma with mucin secretion or mixed adenosquamous carcinomas or adenocarcinoma [3]. Such classification has prognostic significance as majority of squamous cell carcinoma cases with mucin secretion are seen in younger patients [3], [9] and it runs an aggressive clinical course and has poorer prognosis as compared to squamous cell carcinoma or adenocarcinoma [3],[4],[9],[10].

The primary objective of our study is to find out prevalence of mucin secretion in cervical carcinoma by mucin histochemistry with special reference to squamous cell carcinoma (diagnosed by H&E stain) and to find the relative incidence of invasive carcinoma on the basis of H&E stain versus mucin stains.

Materials And Methods

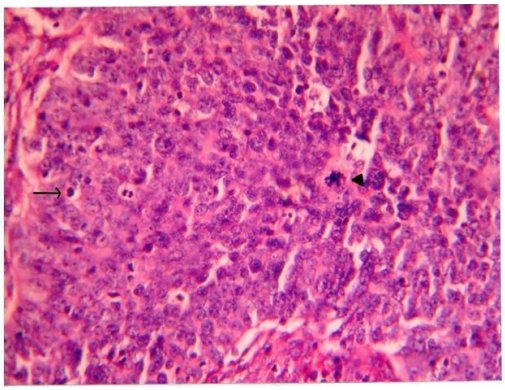
The present study was conducted from January 2006 to December 2009 in the Department of Pathology at Adesh Institute of Medical Sciences and Research, Bathinda. Two hundred and twenty three cases of carcinoma cervix were studied. The tissues were fixed by using 10% formalin and processed through alcohol and chloroform to form paraffin blocks. The tissues were sectioned at 4µm thickness and subsequently stained with H&E, DPAS and Alcian blue. Separate tissue controls were put for DPAS and Alcian blue.

The tumors were classified initially on H&E stained sections according to WHO classification [11]. After mucin stain they were re-classified into Fox classification system [9]. 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 labelled as adenocarcinoma. A 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.

Data was analyzed by using descriptive statistical analysis like percentages; while univariate statistical method of Fisher's exact test was used to compare the two methods. p value <0.05 was considered significant at a confidence interval of 95%

Results

The prevalence of the different histopathological types of cervical carcinomas was studied. The tumors were initially classified based upon H&E stain. Squamous cell carcinoma accounted for 201(90.1%) cases. Of these 132(59.2%) cases were moderately differentiated and 62(27.8%) were poorly differentiated [Table/Fig 1](Fig.1). Adenocarcinoma comprised of 12(5.4%) cases. Seven (3.1%) and 3(1.4%) cases were that of undifferentiated and adenosquamous carcinoma respectively as shown in [Table/Fig 2] .



(Table/Fig 1) Showing poorly differentiated squamous cell carcinoma with frequent atypical mitosis (arrow head). (H&E stain).

(Table/Fig 2)Prevalence of various histological types of cervical carcinomas based on H&E stain.

S.No.	Histopathological Type	No. of patients	Percentage
1.	Squamous cell carcinoma -Well differentiated -Moderately differentiated -Poorly differentiated	201 07 132 62	90.1% 3.1% 59.2% 27.8%
2.	Adenocarcinoma	12	5.4%
3.	Adenosquamous carcinoma	03	1.4%
4.	Undifferentiated carcinoma	07	3.1%
Total		223	100.0%

S.No.	Histopathological Type	No. of patients	Percentage
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2.	Adenocarcinoma	12	5.4%
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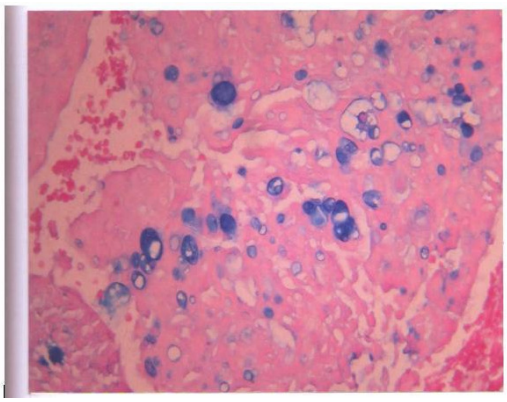
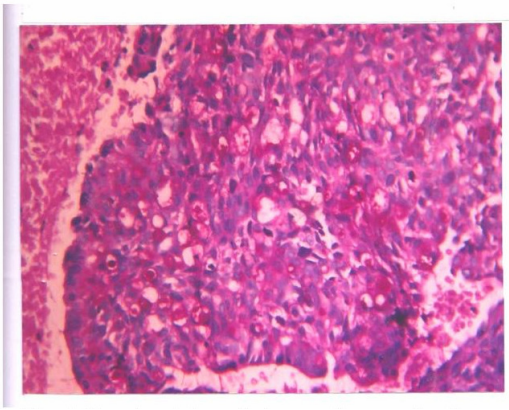
4.	Undifferentiated carcinoma	07	3.1%
Total		223	100.0%

Out of 201 cases diagnosed initially as squamous cell carcinoma with H&E stain, 31(15.4%) cases showed mucin positivity. 16 (12.1%) out of 132 cases of moderately differentiated squamous cell carcinoma and 15 (24.1%) cases of poorly differentiated carcinoma showed mucin positivity [Table/Fig 3],[Table/Fig 4]. No case of well differentiated squamous cell carcinoma showed evidence of mucin. All cases of adenocarcinoma and adenosquamous carcinoma were mucin positive as shown in [Table/Fig 5] .



(Table/Fig 3) Showing intracellular mucin secretion in poorly differentiated squamous cell carcinoma. (Alcian blue stain)



(Table/Fig 4) Showing intracellular mucin secretion in poorly differentiated squamous cell carcinoma. (DPAS Stain)

(Table/Fig 5) Result of mucin histochemistry.

Sr. No.	Histopathological diagnosis	DPAS (No. & percentage of mucin positive cases)	Alcian blue (No. & percentage.of mucin positive cases)
1.	Squamous cell carcinoma -Well differentiated -Moderately differentiated -Poorly differentiated	31(15.4%) - 16(12.1%) 15(24.1%)	31(15.4%) - 16(12.1%) 15(24.1%)
2.	Adenocarcinoma	12(100%)	12(100%)
3.	Adenosquamous carcinoma	03(100%)	03(100%)
4.	Undifferentiated carcinoma	-	-
Total		46(20.6%)	46(20.6%)

Sr. No.	Histopathological diagnosis	DPAS (No. & percentage of mucin positive cases)	Alcian blue (No. & percentage.of mucin positive cases)
1.	Squamous cell carcinoma -Well differentiated -Moderately differentiated -Poorly differentiated	31(15.4%) - 16(12.1%) 15(24.1%)	31(15.4%) - 16(12.1%) 15(24.1%)
2.	Adenocarcinoma	12(100%)	12(100%)
3.	Adenosquamous carcinoma	03(100%)	03(100%)
4.	Undifferentiated carcinoma	-	-
Total		46(20.6%)	46(20.6%)

The relative incidence of the different histopathological types of cervical carcinoma with H&E stain versus mucin stains (Fox classification). This shows that the number of squamous cell carcinoma decreased from 201 (90.1%) to 170 (76.2%). Out of 31 cases 29 (13.0%) cases were re-classified as squamous cell carcinoma with mucin secretion and two cases were reclassified as adenosquamous carcinoma as shown in [Table/Fig 6].

(Table/Fig 6) Classification and comparison of relative incidence of histopathological types of carcinoma cervix based on H&E stain and mucin stains.

Sr. No.	Histopathological diagnosis	H&E stain	Mucin stains	P value*
		No. of cases and percentage	No. of cases and percentage	
1.	Squamous cell carcinoma	201(90.1%)	170(76.2%)	<0.001ǂ
2.	Adenocarcinoma	12(5.4%)	12(5.4%)	1.0
3.	Adenosquamous carcinoma	03(1.4%)	05(2.2%)	0.45
4	Undifferentiated carcinoma	07(3.1%)	07(3.1%)	1.0
5.	Squamous cell carcinoma with mucin secretion	-	29(13.0%)	<0.001ǂ

*** After applying Fisher's exact test. ǂ significant p value of mucin stains in comparison to H&E stain**

Discussion

Worldwide, cervical cancer is the fifth most deadly cancer in women [12].It affects about 16 per 100,000 women per year [13]. In developed countries like United States endometrial carcinoma has taken over due to extensive and successful screening by Pap smear [14]. Classification of invasive carcinoma given by Buckely and Fox is most widely accepted [15]. Benda et al. [4] were the first to demonstrate the importance of mucin secretion in cervical cancer which was confirmed later on [1], [9], [10]. 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. A similar variation in tumor is seen in other anatomical sites with epithelial junction, such as anal canal and gastroesophageal junction. Poorly differentiated neoplasms render more complexity by the fact that a single tumor can exhibit features suggesting an origin from multiple cell types. Mucin stain is integral for correct classification of the tumors [17].

In our study assessment of cervical cancer was done on H&E stain alone and then on both H&E and mucin stains. The results showed that majority of cases were that of squamous cell carcinoma i.e. 201(90.1%) out of 223 cases on H&E stain. After application of mucin stains the number of cases of squamous cell carcinoma was reduced to 170(76.2%). Buckley et al. [9] observed that number of squamous cell carcinoma changed from 187(76.0%) to 152(61.7%) and Misra et al. [7] found that the number of squamous cell carcinoma decreased to75 (76.5%) from 90(91.8%) after applying mucin stains. Squamous cell carcinomas with mucin secretion was the second in list, cases accounting to 29(13%). Misra et al. [7] reported 12 (12.3%) cases of squamous cell carcinoma with mucin secretion out of 98 cases originally reported as squamous cell carcinoma. Other authors have reported cases of squamous cell carcinoma with mucin secretion to be 20%, 7.8% and 8.5% respectively [5], [9], [18]**.** In the present study mucin secretion was mostly seen in poorly and moderately differentiated squamous cell carcinoma, which is in accordance with other studies [7], [8]. None of the well differentiated squamous cell carcinomas showed mucin positivity. Other studies have also reported a similar observation [4], [9], [18], [20].

In the present study we have been able to identify squamous cell carcinoma with mucin secretion from the subgroup of moderately and poorly differentiated squamous cell carcinomas. Identification of squamous cell carcinoma with mucin secretion should be considered separately from pure squamous cell carcinoma and pure adenocarcinoma because such neoplasms runs an aggressive clinical course and is associated with worse prognosis as compared to their squamous and adeno counterparts [4], [9], [10]. Furthermore these tumors occur with increased frequency in women aged less than 40 years and account for poor prognosis in young patients who present with rapidly metastasizing tumors [4],[9].

The survival in mixed tumors was significantly worse than with squamous

cell cancers (P= 0.006), 5-year survival rates being 52% and 75% respectively [10]. Poorer prognosis of mixed carcinomas is because of high incidence of myometrial invasion, pelvic node metastasis and vascular invasion by tumor [9], (10].

Conclusion

The application of mucin stains should be done routinely on moderately and poorly differentiated squamous cell carcinomas to detect cases of carcinoma with evidence of mucin secretion which can be missed on routine haematoxylin and eosin stain.

Tumors so identified are likely to have a more aggressive clinical course associated with poorer survival when compared to non-mucin-producing squamous carcinomas. This is a cost effective investigation with a great bearing on the ultimate treatment and prognosis of the patient.

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