
Utility of PAS and Alcian Blue Stains in Carcinoma Cervix

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Abstract

The present study utilizes stains for mucin preferably PAS and Alcian blue stains to show the classification of invasive carcinoma of uterine cervix are either squamous cell carcinoma or adenocarcinoma. One hundred representative of invasive carcinoma of cervix were undertaken for the study at pathology department of VNGMC from 2011 to 2015. The section were stained with Haematoxyline & Eosin, PAS with & without diastase and alcian blue stain. The histopathological diagnosis was done before and after inclusion of mucin stain. The conventional view is that 90 to 95% of the invasive carcinomas of uterine cervix are squamous cell carcinomas and remaining are adenocarcinomas but in reality this view is incorrect as only 70% of the cervical carcinomas are purely squamous. This change is due to inclusion of mucin stain as a part of routine histopathological examination. These stains are useful in separation of adenocarcinomas into four histological variants.

Keywords: PAS, Alcian blue, carcinoma

1. Introduction

The commonest carcinoma of female genital tract and ranks second among malignancies in females is Carcinoma cervix, breast carcinoma being the most common. No form of cancer better documents remarkable effects of prevention, early diagnosis and curative therapy on mortality rate than carcinoma cervix. Invasive cervical carcinomas have been divided into various categories based on their histological appearance and staining characteristics [1].

Preclinical invasive cancer refers to early cervical cancer, with minimal stromal invasion, often without any symptoms or clinical features. As the stromal invasion progresses, the disease becomes clinically obvious, revealing several growth patterns visible on speculum examination. Histologically 90-95% of invasive cervical cancers are squamous cell cancers; adenocarcinoma constitutes less than 5% of cervical cancers in most developing countries [2].

Routine application of stains for mucin, preferably PAS (Periodic acid schiff) and alcian blue has shown that classification of carcinomas of cervix in H & E stained section gives incomplete and erroneous diagnosis and many mucus secreting neoplasms escape the detection unless mucin stain is applied. Routine application of stains

for mucin has shown that 20 to 30 % of the cervical tumors regarded as being squamous cell carcinomas have to be reclassified either as poorly differentiated adenocarcinomas or as mixed adenocarcinomas. This reclassification is important because mucus secreting neoplasms which morphologically resemble pure squamous cell carcinomas are more aggressive and have worse prognosis than their purely squamous counterparts [3]. The present study carried out at Shri. V.N. Government Medical College, Yavatmal and envisaged with an intention to reexamine H & E stained section and those blocks which were regarded as most representative were recut to provide section for mucin and glycogen stain.

2. Experimental Methodology

The present study was carried out at department of pathology, Shri V. N. Government Medical College, Yavatmal, Maharashtra. The study was design to show the utility of PAS and Alcian Blue staining in differentiating adenocarcinomas from squamous cell carcinomas.

One hundred blocks representatives of the invasive carcinoma of cervix were recut at pathology department of VNGMC Yavatmal from 2011 to 2015. The

sections were stained with following stains. 1. Haematoxylline and Eosin, 2. PAS with and without diastase, 3. Alcian blue stain. The original histopathological diagnosis of 100 cervical carcinomas before the introduction of mucin stain was compared with diagnosis after the inclusion of mucin stain. Each section was stained with sufficient care to afford definite conclusion regarding presence or absence of mucin. All the section were reviewed and examined.

Tumours which showed well defined, squamous pattern of growth and on special stains showed no mucin production or in less than in 5% of tumour volume were classified as squamous cell carcinoma. They were further categorized as well, moderately and poorly differentiated tumours.

Tumours with no evidence of squamous differentiation but showing either formation of glandular structures or widespread mucin secretion that is in at least 75% of tumour cells were classified as adenocarcinomas.

The diagnosis of adenosquamous carcinoma was made only when the neoplasm contained both squamous and adenomatous elements and the minor component comprised at least one third of the total. Well differentiated carcinoma was recognized by presence of well differentiated glandular element and keratinizing squamous cell carcinoma. The less well differentiated carcinomas were morphologically less distinct but there was mucin secretion in >30% of the tumour volume. Mucin secreting cells were either nonspecific or signet ring like.

Diagnosis of squamous cell carcinoma with mucin secretion was made when mucin secretion in these tumours was >5% but <30% of the tumour volume.

3. Observation and Result

A total 100 cases were studied. Staining was done first with H&E alone and then with special stains such as PAS and alcian blue stain. All the cases were classified in various categories as shown in table 1 and 2.

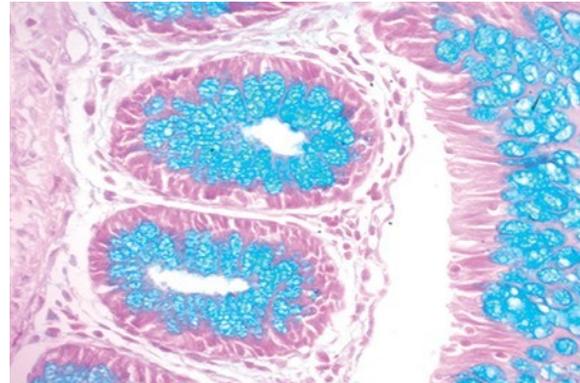


Fig 1: Alcian Blue stain- Carcinoma Cervix

Table 1: The original histopathological diagnosis and the number of cases

| Original diagnosis | Number of cases |
|-----------------------------|-----------------|
| Squamous cell carcinomas | 92 |
| Adenocarcinomas | 3 |
| Adenosquamous carcinomas | 3 |
| Undifferentiated carcinomas | 2 |

Table 2: The revised histopathological diagnosis after inclusion of mucin stains

| Revised diagnosis | Number of cases |
|-----------------------------|-----------------|
| Squamous cell carcinomas | 85 |
| Adenocarcinomas | 6 |
| Adenosquamous carcinomas | 9 |
| Undifferentiated carcinomas | 0 |

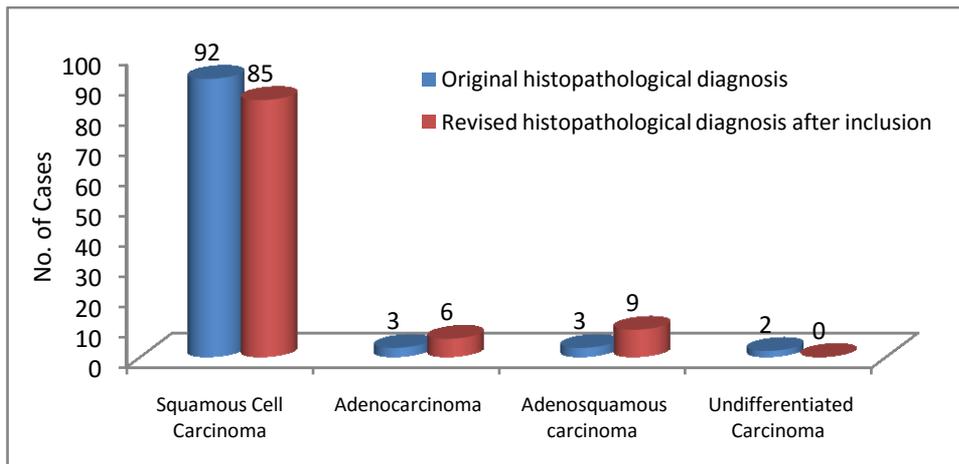


Fig 2: Diagrammatic representation of histopathological diagnosis before and after the inclusion of mucin stains

4. Discussion

Histologically, approximately 90-95% of invasive cervical cancers arising from the uterine cervix in developing countries are squamous cell cancers and 2 to 8% are adenocarcinomas[3].

For several decades, cervical carcinoma ranked first among the malignancies of female genital tract. In most of the countries it still remains the most common malignancy of female genital tract, though in developed countries like united state, endometrial carcinoma has

taken over [4]. Classification of invasive carcinoma cervix and the criteria defined for it, been changing frequently over the past few decades. Classification as given by Buckley and Fox is most widely accepted [1].

Broadly the categories routinely considered are squamous cell carcinoma, adenocarcinoma and mixed carcinomas (adenosquamous carcinoma), since other lesions are quite rare. The lesions which are well differentiated, usually do not pose any diagnostic problem, but the lesions which appear to be poorly differentiated squamous cell carcinomas on H&E stain, may turn out to be poorly differentiated adenocarcinomas or mixed carcinomas, when staining for mucin is carried out, depending upon the amount of mucin present.

This fact emphasizes the importance of including mucin stains as a routine for the diagnosis of cervical carcinoma. The present study was also carried out to access the accuracy of this fact in Indian context. Criteria for diagnosis were followed as given by Buckley, Beards and Fox [5]. In our study, it has been observed that squamous cell carcinomas form the major proportion of case studied, number being 85 (85%) out of 100 cases. Mixed carcinomas were second in list, cases accounting to 9 in number (9%) and adenocarcinoma were the last common out of these three categories, the number being only 6 (6%).

As far as incidence of mixed carcinomas is concerned, our results are in accordance with other workers who have reported the incidence ranging from 3.6 to 6% [6,7]. Some author reported higher incidence of mixed carcinomas that is 8 to 9.5% [8-10]. Probably these studies were carried out in middle of the century or earlier and by that time the criteria for diagnosis of various histological types had not been very well defined. On the other hand some workers reported quite high incidence of mixed carcinomas ranging from 16-26% [5,11-13] possibly due to inclusion of small number of cases in their studies.

Two well established staining techniques were used in the study that is PAS with diastase and alcian blue. On the whole we found alcian blue stain preferable due to greater specificity and intensity of staining. PAS with diastase also gave excellent results, the only drawback being keratin also taking up the stain giving false positive results; though on careful examination it was found that the positivity shown by keratin was granular, quite different from block or diffuses positivity shown by mucin.

5. Conclusion

PAS and Alcian blue stains are useful in demonstrating mucin production in cervical carcinomas. PAS stains gives positive reaction with glycogen or neutral polysaccharides but alcian blue stain is more specific as it

reflects the content of mucopolysachharides. So these stains are useful in separation of adenocarcinomas into four histological variants.

Thus the study concluded with 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&E staining alone.

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