p16 Expression Pattern in Different Morphological Variants of Cervical Adenocarcinoma in Bangladeshi Women

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Abstract

Background: The incidence of cervical adenocarcinoma has been reported to be increasing. Cervical carcinoma is the second most common cancer as well as the second highest cause of cancer-related deaths among Bangladeshi females. Due to the poor prognosis of cervical adenocarcinoma, early diagnosis is crucial to ensure patient management and survival. High risk Human Papilloma Virus (HPV) is the predominant etiologic agent in cervical premalignant and malignant lesions including adenocarcinoma of cervix**.** Association of HPV in cervical adenocarcinoma including its different morphological types has not been explored in Bangladesh.

Objective: The study objective was to diagnose the morphological variants of cervical adenocarcinoma as well as to investigate their expression pattern of p16 immunostain as a surrogate biomarker of high-risk HPV infection in Bangladeshi women.

Methods: This retrospective observational study was conducted with the paraffin-embedded tissue blocks of 40 cases of cervical adenocarcinoma diagnosed histologically from January 2014 to February 2017. The morphological variants of cervical adenocarcinoma were determined based on routine H&E stain. Immunostaining was done in all cases with p16 antibody.

Results: Of the 40 cases of cervical adenocarcinoma, 18(45%) were the usual endocervical adenocarcinoma, whereas 11 were endometrioid variant, 7 villoglandular variant, 2 serous and 2 clear cell variant. Histologically, 68% of cervical adenocarcinomas were well differentiated, 27% moderately and 5% poorly differentiated. All 40 cases of cervical adenocarcinoma, irrespective of the morphological variant, exhibited p16 immunoreactivity with variable intensity and distribution pattern (focal versus diffuse). Majority of the endocervical and endometrioid variants as well as the serous and clear cell ones showed diffuse (88%) positivity for p16 with strong intensity (90%).

Conclusion: Immunostaining with p16 antibody can be used as a surrogate biomarker to explore the association of high-risk HPV with cervical adenocarcinoma.

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Keywords: Cervical cancer, Adenocarcinoma, Morphological variants, Expression pattern of p16

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Introduction

Cervical carcinoma is the fourth most common cancer in women and fourth most common cause of cancer related deaths in the world during 2020.[1] In Bangladesh, it is the second most common cancer and the second highest cause of cancer related deaths among women.[2] The incidence of cervical squamous cell carcinoma has steadily declined over the last four decades in many developed countries mainly due to cytological screening programs with Papanicolaou test.[3] However, recent studies have reported a rising trend in the incidence of cervical adenocarcinoma worldwide.[4,5]

Currently cervical adenocarcinoma constitutes 10-25% of total cervical carcinomas in developed countries, even though three decades ago the incidence was 5-10%.[6] A similar trend was also observed at the department of pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka,

Diagnosis of cervical adenocarcinoma is clinically very important because of its poor prognosis owing to less sensitivity to radiotherapy and chemotherapy in comparison to squamous cell carcinoma.[7,8] Carcinomas that arise in the endocervix usually display variable histomorphology sometimes leading to diagnostic difficulty.[9,10,11] However, meticulous assessment of cells and growth pattern with hematoxylin and eosin (H&E) stain is sufficient in determining the morphological variant of cervical adenocarcinoma, while sometimes mucin stains, like periodic acid Schiff (PAS), may help.

It is well-established that high-risk Human Papilloma Virus (HPV) is the predominant etiologic agent in cervical premalignant and malignant lesions.[12] More than 90% of cervical adenocarcinomas are caused by high-risk HPV, most commonly types 18, 16 and 45.[6] Papillomavirus is a double-stranded DNA virus encased in a 72-sided icosahedral protein capsid. More than 120 types of HPV have been identified, which are divided into high-risk, intermediate-risk, and low-risk types. The persistent high-risk type HPV infection of the cervical epithelium appears to trigger neoplastic progression.[13,14]

The majority of HPV detection tests rely on DNA-based detection of a portion of the gene, but these tests cannot differentiate between transient and potentially transforming infection.[15] One of the alternatives to HPV DNA testing (suggested at the consensus report by the working group in the 2006 meeting of the European Organization on Genital Infection and Neoplasia) is detection of cellular proteins that are over expressed by HPV-infected cells; one such protein is the $P16^{INK4a}$ gene product.[15] Cellular expression of p16 protein with immunohistochemistry has been validated by several biomedical researches as a surrogate biomarker of high-risk HPV infection in cervical premalignant and malignant lesions.[16] Although HPV genomic study is more specific to establish its association with a cancer, it is technically challenging in formalin-fixed paraffin-embedded tissue. Thus, p16 immunostain is often used in cases of cervical cancers and precancerous lesions to identify association with high risk HPV.

p16 is a tumor suppressor protein that inhibits the cyclin-dependent kinases (CDK) 4 and 6, which regulate the G1 checkpoint. The CDK phosphorylate the Rb protein, which results in conformational change and the release of E2F from Rb protein. Inactivation of either the p16 or Rb function allows the cell to enter the S phase after only a brief pause at the G1checkpoint. Rb inactivation has been shown to be reciprocal to p16 expression.[17] Moreover, the interrelationship

between immunohistochemical expression of p16 and infection with different HPV subtypes in genital and cervical preneoplastic and neoplastic lesions is well established and p16 is considered as surrogate marker of Human Papilloma virus infection.[18–20]

Association of HPV in different morphological types of adenocarcinoma in Bangladesh has not been studied yet. This study was undertaken to evaluate in detail the morphological types of cervical adenocarcinoma, as well as to investigate the expression pattern of p16 immunostain as a surrogate marker of high-risk HPV infection.

Methods

This is a retrospective observational study carried out at the Department of Pathology, Bangabandhu Sheikh Mujib Medical University (BSMMU) from July 2015 over a period of two years. After approval from the Institutional Review Board of BSMMU, cases of adenocarcinoma of the cervix diagnosed histologically during January 2014 - February 2017 at Pathology Department, BSMMU, and a private histopathology diagnostic center were selected for the study. Paraffin-embedded tissue blocks of 40 cases of cervical adenocarcinoma with the presence of adequate tumor tissue were retrieved from the respective archives. The cases were comprised of six hysterectomy specimens, two Loop electrosurgical excision procedures (LEEP), and 32 colposcopy-guided cervical biopsy specimens. The study did not require any physical intervention or any personal information other than the age and the histological diagnosis, and therefore, patient consent was not necessary.

Routine tissue sections stained with H&E and PAS from each case were reviewed microscopically to confirm the histological diagnosis as well as to determine the morphological variant of the respective cervical adenocarcinoma.

For immunostaining, 5 µm thick sections were cut, deparaffinized with xylene and rehydrated through a graded series of alcohol. For antigen retrieval, the samples were treated with Dako Target Retrieval solution (pH 9.0 for p16). Solutions were taken in coplin jar and pre-heated in the water bath at 65°C. Then slides were kept in this solution and heated in the water bath at 95-99°C for 30-40 minutes. Subsequently, the sections were stained with p16INK4a antibody (abcam, England). Immunostaining was done manually following the avidin-biotin-peroxidase staining method. Positive control was taken from sections of invasive squamous cell carcinoma of cervix. Expression of p16 was considered positive based on the obvious chestnut-brown color in the nucleus and cytoplasm. The intensity of the reaction was scored as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). p16 immuno-reactivity was scored as negative (no immunoreactive cell), focally positive (<50% cells immunoreactive), or diffusely positive (>50% cells immunoreactive) based on a previous report.[21]

Descriptive statistics were used for statistical analysis. The mean values were calculated for continuous variables. The quantitative observations were indicated by frequencies and percentages.

Results

The age range of the patients were 30 - 69 years, with the mean age of 45 years. About 75% of the patients were more than 40 years of age (Table I).

Table I: Age of the patients (n=40)

Age range	Number of Cases (%)
30-39	10 (25%)
40-49	14 (35%)
50-59	12 (30%)
60-69	4 (10%)

Microscopic evaluation of H&E and PAS-stained sections revealed the following morphologic variants of the 40 cases of cervical adenocarcinomas: 18 (45%) usual endocervical type, 11 (27.5%)endometrioid, seven (17.5%) villoglandular, two (5%) serous, and 2 (5%) clear cell variants (Figure 1).

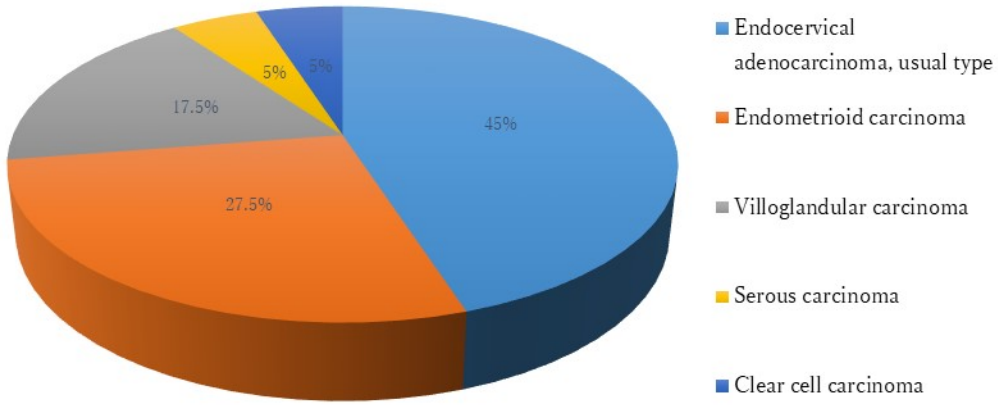

Figure 1**.** Distribution of morphological variants of cervical adenocarcinoma

Histological grading of the morphological variants were presented in Figure 2. It shows that the vast majority (68%) of the cases to be well differentiated, including the endocervical adenocarcinomas, endometrioid as well as villoglandular variants (Figure 2). Both the serous variants were moderately differentiated, while both the clear cell variants were poorly differentiated.

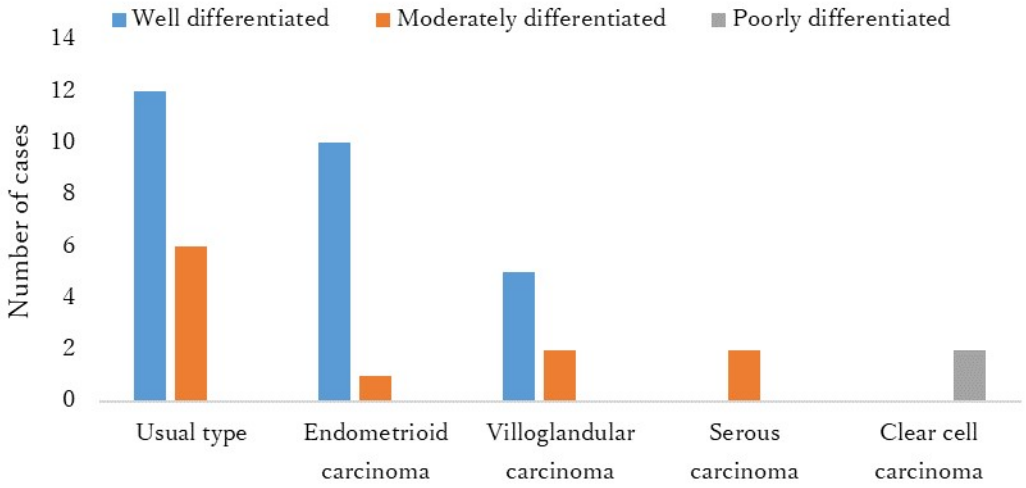

Figure 2. Grading of morphological variants of cervical adenocarcinoma

Expression of p16 in different morphological variants of cervical adenocarcinoma:
P16 immunoreactivity was present in all cases of cervical adenocarcinoma with variable intensity (Table II) and distribution pattern (Table III). Most of the endocervical and endometrioid variants were strongly p16 positive, so were both of the clear cell variants. The rest of the cases showed moderate to weak positivity (Figure 3).

Table II: p16 positivity in different histological types of cervical adenocarcinoma

Types of cervical adenocarcinoma	Number (%)	Strong p16 positivity	Moderate p16 positivity	Weak p16 positivity
Endocervical	18(45%)	11(61%)	5(27%)	2(11%)
Endometrioid	11(27.5%)	9(81%)	2(18%)	0
Villoglandular	7(17.5%)	4(57%)	2(29%)	1(14%)
Serous	2(5%)	1(50%)	1(50%)	0
Clear cell type	2(5%)	2(100%)	0	0

Table III showed p16 positivity distribution pattern, i.e., diffuse or focal, in different variants of cervical adenocarcinoma. It is evident that most of the cases (88%) were diffusely positive for p16.

Table III: p16 positivity distribution pattern in different variants of cervical adenocarcinoma

Variants of cervical adenocarcinoma	Distribution pattern of p16 positivity	
	Focal (<50% cells)	Diffuse (≥50% cells)
Endocervical (18 cases)	3 (17%)	15 (83%)
Endometrioid (11 cases)	1 (9%)	10 (91%)
Villoglandular (7 cases)	1 (15%)	6 (85%)
Serous (2 cases)	0	2 (100%)
Clear cell (2 cases)	0	2 (100%)
Total (40 cases)	5 (12.5%)	35 (87.5%)

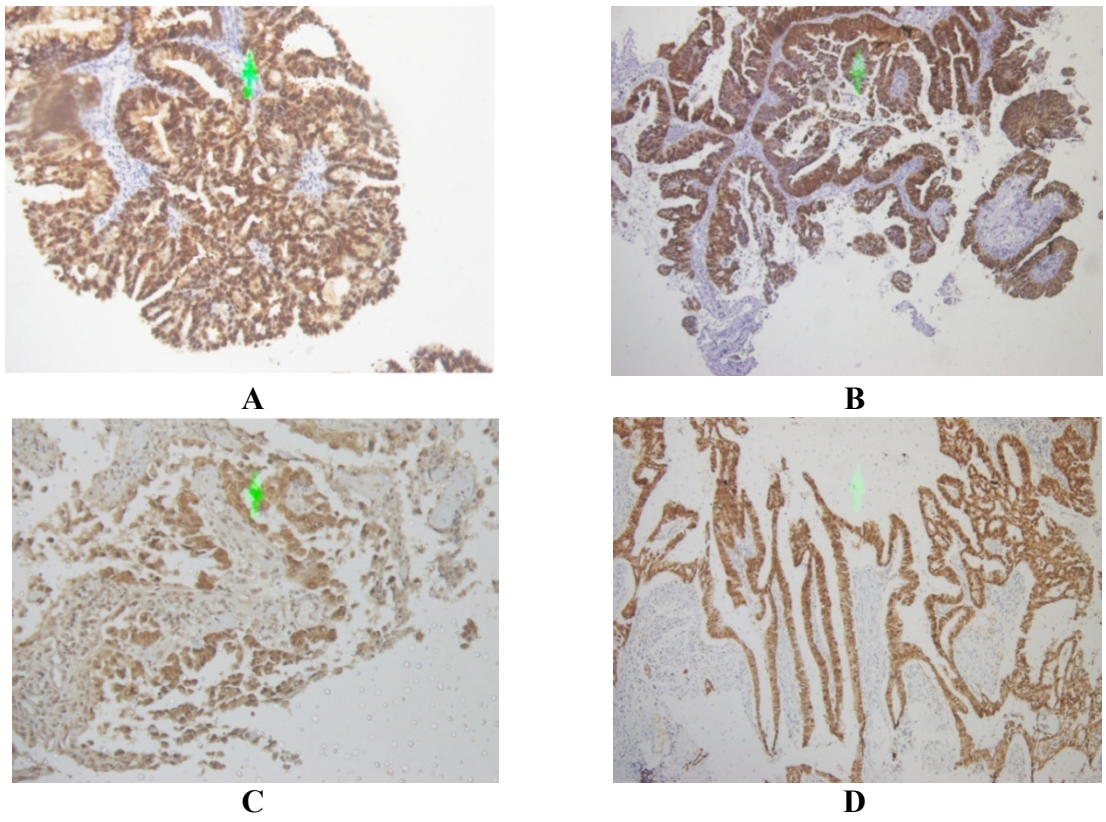

Figure 3**.** Photomicrograph showing grading of morphological variants of cervical adenocarcinoma. A. p16 positivity in endocervical adenocarcinoma (Case no. 30, p16 immunostain, x200). B. Strong and diffuse p16 positivity in endometroid adenocarcinoma (Case no. 27, p16 immunostain, x100). C. Positive nuclear staining of p16 of the tumor cells by Villoglandular type cervical adenocarcinoma (Case no. 37, p16, immunostain, x100). D. Positive nuclear staining of p16 of the tumor cells by serous type cervical adenocarcinoma (Case no. 9 p16, immunostain, x200).

Discussion

The incidence of adenocarcinoma of cervix has been reported to be increasing in many parts of the world including Bangladesh.[4,5] The morphological diversity of cervical adenocarcinoma is well established.[9,10,11] So, thorough knowledge of the variable morphology is essential to make a definitive diagnosis of cervical adenocarcinoma to ensure patient's better treatment and prognosis. This study determined the morphological variations of 40 histologically diagnosed cases of cervical adenocarcinoma and also investigated the expression pattern of p16 immunostain as a surrogate biomarker of high-risk HPV infection.

Out of 40 cases, endocervical adenocarcinoma, usual type was the most common type (18 cases, 45%). Most of these tumors were well to moderately differentiated, characterized by papillary and glandular structures. Wilbur et al.[6] stated that endocervical adenocarcinoma account for 90% of all adenocarcinoma of cervix, and they are mostly well to moderately differentiated. According to Kindelberger et al.[22] cytoplasm of the cells of cervical adenocarcinoma may be eosinophilic,

mucinous appearing or a mixture of these. In this study, the number of endocervical adenocarcinoma, usual type was less than expected (90% versus 45%), possibly because the sample size was small.

The endometrioid variants presented with more crowded and stratified cells resembling endometrial epithelium. Eleven cases (27.5%) of cervical adenocarcinoma were of endometrioid carcinoma in this study, representing a much greater percentage than those reported in literatures. According to Wilbur et al.[5]endometrioid variants are rare accounting for not more than 5% of all cervical adenocarcinoma. However, the exact occurrence of endometrioid variant is not well established, because the morphological features sometimes overlap and subjective variation plays a significant role in making the histological diagnosis.[22]

Among the 40 cases, 7 (17.5%) were diagnosed as villoglandular carcinoma showing villous-papillary architecture. Though this tumor has been reported as uncommon, it is the third most common variant of cervical adenocarcinoma. Serous carcinoma and clear cell variants, reportedly high grade in nature (moderate to poor) were the least common, each representing 5% of the total.

Recent research is focused on the development of objective biomarkers that can distinguish transforming HPV infections from productive HPV infections and also to predict disease severity. The cellular tumor suppressor protein p16INK4a (p16) has been identified as a biomarker for transforming HPV infections. p16 is expressed in HPV-associated lesions like low-grade cervical intraepithelial neoplasia (CIN), and in a high percentage of high-grade CIN. So the diagnostic application of p16 in cervical pathology has been investigated also. Moreover, in glandular cervical lesions, p16 could be also useful with appropriate markers panel, to detect the type and site of the lesion.[23]

In the present study, it was observed that all cases of cervical adenocarcinoma were positive with p16 immunostain. Out of 40 cases, 68% showed strong p16 positivity including 11cases (around two-thirds) of endocervical adenocarcinoma and 10 cases (all except one) of endometrioid carcinoma. Strong p16 positivity was also observed in both cases of clear cell carcinoma.

A total of 35 out of 40 cases (88%) showed diffuse distribution pattern of p16 including all cases of both clear cell carcinoma and serous carcinoma. On the other hand, focal positivity of p16 was seen in 3 cases of endocervical variant and one each of villoglandular and endometrioid carcinoma. Klaes et al.[24] showed that six (86%) out of seven adenocarcinoma cases show diffuse p16 positivity where one case was negative for p16 (14%). The study conducted by Izadi-Mood et al.[17] showed that two (25%) out of 8 cervical adenocarcinoma cases were p16 negative. Three (37.5%) cases exhibited strong p16 staining whereas 2 were moderate and 1 was weak in p16 staining. Caponio et al.[23] showed in their study that 5 out of 6adenocarcinomas of endocervical origin showed diffuse positivity for p16, whereas one case was only focally positive.

Both cases of clear cell carcinoma were diffusely positive for p16 in this study. Park et al.[25] reported in their study that all nine cases of clear cell carcinoma showed p16 positivity including four cases with diffuse p16 staining whereas 3 with patchy and 2 with focal.

Conclusion

Despite variable morphology, all the 40 cases of cervical adenocarcinoma were positive

with p16 immunostain indicating infection with high-risk HPV. Among them, 88% cases showed diffuse positivity for p16, while the remaining cases were focally positive. As a surrogate biomarker for high-risk HPV, the study findings implicate the association of high-risk HPV in the vast majority of cervical adenocarcinomas, and confirm the current epidemiological data that high-risk HPV infection is the main etiological agent of cervical adenocarcinoma. However, the study has several limitations including lack of correlation of p16 positivity with HPV genomic data, a small sample size, and retrospective nature of the study. The results recommend that a prospective study with larger sample size. HPV genomic study will be of much value to ratify the effectiveness of currently available vaccines against HPV in Bangladesh.

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