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''Role of P16 and Ki67 expression in premalignant and malignant lesion of cervix''

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Abstract: Study Design: *Prospective study.*
Purpose: *To asses the role and positivity of P16 and Ki67 in premalignant and malignant lesion of cervix*
Background: *Cervical cancer is the 3rd most common cancer world-wise and 13thmost cause of cancer mortality[1]. The majority of cases occurred in less developed countries and developing countries which usually presented late.*
Squamous cell carcinoma is the most common histologic type accounting for approximately 80% followed by adenocarcinoma (15%). Adenosquamous and neuroendocrine tumors are very rare and accounts for only 5% of total cases. All of these cases are associated with high risk HPV.
No form of cancer better documents the remarkable benefits of effective screening early diagnosis and curative therapy than cancer of cervix.
Methods*: We performed a prospective study on 50 patients with abnormal cytology or with high risk HPV as an indication for colposcopy and biopsy or direct tissue biopsy from gross cervical lesion. All patients who came to gynaecology clinic of Rajendra institute of medical sciences between November 2016 to October 2017 were included in this study.*
Results*: Out of 50 patients, there were 25 (50%)of LSIL, 15 (30%) of HSIL and 10 (20%) of invasive cancer evaluated histo-pathologically. Age of patients varied from 20 to 78 years with mean age 45.*
On Immunohistochemical staining, 24% of LSIL, 73% of HSIL AND 100% of invasive cancer showed Ki positivity; while 12% of LSIL, 80% OF HSIL and 100 % of invasive cancer showed P16 positivity.
Conclusions*: Association of expressions of P16 and Ki 67 and severity of cervical lesion were found to be significant. So expression of these markers will be useful in grading cervical lesion with equivocal histopathological finding.*
Key words: *P16, Ki 67,Malignant,Screeing,Invasive*

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I. Introduction

Cervical cancer is the 3rd most common cancer world-wise with an estimated 530000 new cases in 2008, out of which more than half are fatal in women[1]. 50 years ago, carcinoma of cervix was the leading cause of cancer death in women in united states, but the death rate has declined by 2/3rdto its present rank as 13thcause of cancer mortality[1]. The majority of cases occurred in less developed countries and developing countries which usually presented late.

Squamous cell carcinoma is the most common histologic type accounting for approximately 80% followed by adenocarcinoma (15%). Adenosquamous and neuroendocrine tumors are very rare and accounts for only 5% of total cases. All of these cases are associated with high risk HPV. Progression time is shorter in adenosquamous and neuroendocrine tumours to undergo from premalignant to malignant one and also have poor prognosis.

No form of cancer better documents the remarkable benefits of effective screening early diagnosis and curative therapy than cancer of cervix.

Purpose: To asses the role and positivity of P16 and Ki67 in premalignant and malignant lesion of cervix

II. Materials and Method

This study was done in department of pathology (Rajendra institute of medical sciences) after approval of institutional ethics committee. It was a prospective type of study. All cervical punch biopsies and postoperative biopsy materials were taken in this study.

Inclusion criteria: women who came to gynaecology clinic of Rajendra institute of medical sciences between November 2016 to October 2017 with abnormal cytology or with high risk HPV as an indication for colposcopy and biopsy or direct tissue biopsy from gross cervical lesion.

Exclusion criteria : Those who had inadequate cervical tissue for histological diagnosis or unavailable tissue block to process with immunohistochemical study.

Only cases with definitive diagnosis were included in the study. Cases with inconclusive diagnosis were excluded. All cases were reviewed by my guide and for each case, a representative block were selected and immunohistochemical stains with P16 and Ki 67 were performed. Nuclear as well as cytoplasmic staining were considered positive.

Cervical tissue were processed and stained Hematoxylin and eosin followed by with P16 and Ki 67 immunohistochemical stain by usual method.

Result of Ki67 immunostaining are interpreted as nuclear stain positivity and scoring is assessed as follow

Score 0- no positive nuclei
Score 1- < 5% positive nuclei
Score 2- 5%-30% positive nuclei
Score 3- > 30% positive nuclei

P16 scoring was calculated by studying a minimum of 5. Fields in the highest labelled area and scoring was assessed as follow:

Score 0- no positive nuclei
Score 1- < 5% positive nuclei
Score 2- 5%-30% positive nuclei
Score 3- > 30% positive nuclei

Hematoxylin and eosin stained pathological slides of all cases were reviewed by an another pathologist trained in gynaecologic pathology. Immunohistochemical staining was performed on 3 mm section of formalin fixed paraffin embedded tissue section.

Expression of immune-stained slides was interpreted independently by two authors under a transmission light microscope. Positive P16 expression was interpreted with a diffuse staining in both nuclear and cytoplasm of basal parabasal with or without superficial cells.

Positive Ki 67 expression was diagnosed with nuclear stain in the intermediate and superficial cells. Ki 67 staining in basal and parabasal cells was diagnosed as negative.

Number and percentage were used to describe categorical variables; mean and range were used for continuous variable. The expression of P16 and Ki 67 were compared using chi square chart. P value < 0.005 was considered significant. Data were analysed by using IBM SPSS statistics version 20.

III. Results

There were 50 patients included in this study. Out of 50 patients, there were 25 (50%)of LSIL, 15 (30%) of HSIL and 10 (20%) of invasive cancer evaluated histo-pathologically. Age of patients varied from 20 to 78 years with mean age 45.

On Immunohistochemical staining, 24% of LSIL, 73% of HSIL AND 100% of invasive cancer showed Ki positivity; while 12% of LSIL, 80% OF HSIL and 100 % of invasive cancer showed P16 positivity.

Chi square test P16:

	value	Df	Asympt.sig.
Pearson chi square test	19.834[3]	2	.000
Likelihood ratio	24.043	2	.000
No. of valid cases	50		

Chi square test of Ki 67

	value	Df	Asympt.sig.
Pearson chi square test	19.834[3]	2	.000
Likelihood ratio	24.043	2	.000
No. of valid cases	50		

Chi-square test showed p value <0.01 which showed significant association between histological grade of cervical lesion and immunohistochemical staining.

IV. Discussion

Gold standard for diagnosis of squamous intraepithelial lesions and invasive carcinoma of cervix is histopathology. Sometime, making a diagnosis based on histopathology become difficult whenever there is equivocal histopathological finding.

However previous studies have evaluated immunohistochemical expression of biomarkers in cervical intraepithelial lesions as an adjunct for a diagnosis of cervical squamous intraepithelial lesion and invasive carcinoma. Our study found p16 expression in 100% of invasive cancer, 80% HSIL, only 12% in LSIL. Other previous studies reported p16 in 80% to100% in invasive carcinoma, 45% to 100% in CIN2/3, and 0% to 15% in non-dysplasia (Volgareva et al., 2004; Wang et al.,2004; Murphy et al., 2005; Benevolo et al., 2006; Ishikawa et al., 2006; Queiroz et al., 2006; Hariri & Oster, 2007; Aslani et al., 2013).

The variation of expression rates may be due to less numbers of cases and criteria defining positive expression. Most of other studies which found almost similar rate of p16 expression. Three studies used criteria of nuclear or cytoplasmic staining as positive (Wang et al.,2004; Murphy et al., 2005; Queiroz et al., 2006) while our study required both nuclear and continuous diffuse cytoplasmic staining of the cells in basal and parabasal as positive. One of the two studies had follow-up data which showed persistent or progressive CIN lesions in a large number of patients (23 cases progressed and 6 cases persisted). The authors even remarked that their high rate of p16 expression in CIN1 might be due to an underestimation of CIN2/3 to CIN1 at the beginning (Hariri & Oster, 2007).

Immunopositivity for Ki67, marker for cell proliferation, linearly increase as the CIN grade is higher (Nam et al., 2008; Kim et al., 2011). Our study found Ki67 expression in 100% of invasive cancer, 73% HSIL, only 24% in LSIL.

These were concordant with previous studies which found Ki67 in 90% to100% in invasive carcinoma, 20% to 70% in CIN2/3, 70% to 90% in CIN1, and 0% to 20% in non- dysplasia (Keating et al., 2001; Agoff et al., 2003; Walts & Bose., 2008; Conesa et al., 2009; Cavalcante et al., 2012; Jackson et al., 2012). The expression of p16 and Ki67 in our study was significant different between CIN2/3 vs CIN1 and Ki67 between invasive carcinoma vs CIN2/3.

The rates of 16 and Ki67 expressions were directly associated with the severity of cervical lesions but should be interpreted cautiously. The possible reason for lower expression (false negative) in cases of ≥ CIN 2 may be caused by low risk-HPV because the affinity of E7 protein of low risk-HPV is much lower than of HR-HPV or the tumor extensively necrotic and decrease detection of HPV Sensitivity was highest at 91.6% when either p16 or Ki67 positive and specificity were highest 96.2% when both markers were positive. The improvement of sensitivity and specificity when both stains were used together was also demonstrated in previous study which showed high sensitivity and specificity or 94% and 90% respectively using both tests (Van et al., 2007).

Limitation: Our study had some limitations. Our study had limited numbers of cases (50), as well had no information regarding HPV infection. A further prospective study with large numbers of cases with more accurate data on HPV infection will be appropriate.

V. Conclusion

Association of expressions of P16 and Ki 67 and severity of cervical lesion were found to be significant. So expression of these markers will be useful in grading cervical lesion with equivocal histopathological finding.

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