Original Research Article

# Expression of Ki 67 and human papilloma virus 16 with risk factors in cervical pre malignant and malignant lesions

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## Abstract

**Introduction:** The present study was conducted to assess immunohistochemical expression of Ki-67 and HPV 16 in premalignant and malignant lesions of cervix. It was also intended to study the risk factors which may be associated with these lesions in Uttarakhand state of India. **Material and Method:** The study including premalignant and malignant lesions of cervix diagnosed over period of one year. Immunohistochemical staining on paraffin blocks for all cases and controls were done for HPV 16 and Ki-67. Ki-67 score was quantified depending on the percentage of positive cells and was statistically analyzed in association with HPV expression. **Results:** The study included 50 cases of premalignant and malignant cervical lesions and 50 controls. 87.2% of the HPV 16 positive cases were also positive for Ki-67 expression which was statistically significantly higher in comparison to controls. Ki-67 expression was negative in all the controls while 90% of cases showed positivity for Ki-67. The malignant lesions of cervix show a significantly higher grade of Ki-67 expression in comparison to premalignant lesions (p value< 0.001). **Conclusion:** Low socioeconomic and education status, smoking (either active or passive), early age of sexual contact and first conception with high parity were associated with increased risk of cervical premalignant and malignant lesions in this region of India. Ki-67 plays an important role in carcinogenesis of cervical cancer in association of HPV16. The increase of immunopositivity of Ki-67 with the severity of lesions suggests its use in stratification of premalignant and malignant lesions of cervix.

**Keywords:** Cervical cancer; Premalignant lesion; Ki-67; Human Papilloma virus

## Introduction

According to GLOBOCAN 2018, worldwide cervical cancer is the 4th most common malignancy among females, with age standardized incidence rate of 13.1 per 100,000 and mortality rate of 6.9 per 100,000 population [1]. However, the occurrence of cervical cancer is higher in Indian females with age standardized rate of 14.7 and mortality of 9.2 per 100,000 population [1]. Human papilloma virus (HPV) infection is amongst the foremost causes of cervical cancer.

The spectrum of morphological lesions caused by HPV cervical infection is large, ranging from normal to precancerouslesions (cervical intraepithelial neoplasia) and finally leading to invasive cancer. Among the 15 high risk HPVs, HPV-16 is the most predominant form and is known to contribute to 60% of the total cervical cancer cases [2].Viral proteins E6 and E7 are the main viral components contributing to carcinogenesis, mainly via interfering with the tumor suppressor genes (p53 and Rb) which leads to uncontrolled cell cycle progression by surpassing the G1 phase arrest. This in turn, alters the expression profiles of proliferation markers such as Ki-67[3]. Ki-67, a nuclear proliferation marker is encoded by MKI 67 gene in humans [4]. In CIN lesions, its expression is proportional to the extent of abnormally proliferating cells in the stratified squamous epithelium [5]. The present study was therefore done to assess the immunohistochemical expression and correlation of Ki-67 with HPV 16 in premalignant and malignant lesions of cervix. It was also intended to study the various risk factors which may be associated with these lesions in Uttarakhand, a north Himalayan state of India.

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## Material and Method

Setting and type of study: Cross sectional prospective study was conducted in the department of Pathology in the Himalayan Institute of Medical Sciences, Dehradun situated in Uttarakhand state of India over a period of 1 year from January 2017 to December 2017 after obtaining written informed consent and approval from institutional ethics committee.

**Sample size:** 100 samples were including for statistical purpose by convenient sampling. 100% enumeration of samples from all eligible study subjects reported during the study period of one year was considered.

**Sample methods:** All consecutive cases diagnosed with cervical premalignant and malignant lesions were included in the study.

**Selection of Subject**

**Inclusion Criteria:** Patients diagnosed with chronic cervicitis, cervical intraepithelial neoplasia (CIN) I, II, III and cervical carcinoma diagnosed on histo-pathological examination of cervical biopsies/ total hysterectomy specimen.

**Exclusion criteria:** Patients of benign neoplasm of cervix including papilloma, condyloms, polyp and leiomyoma.

**Study protocol**

1.All the relevant clinical details along with socio-economic status, dietary habits, smoking status, sexual and obstetrical history were noted for every case after written informed consent in case reporting form.

2.Cervical biopsy tissue/total hysterectomy specimens were received from the department of Gynaecology as per standard procedure.

3.Histopathological tissues were collected and the tissue fixation was performed in 10% formalin solution, grossed and processed according to the Standard Procedure being followed in the department.

4.Histopathological diagnosis of cervical lesions was made according to WHO classification of cervical tumors

5.After histopathological diagnosis, immunohisto-chemical analysis for HPV 16 (Bio Genex, California, USA) and Ki-67 (Bio Genex, California, USA) was performed on paraffin embedded, formalin fixed biopsy sections as per standard procedure and instructions given by manufacturers.

6. HPV 16 immunostained slides were reported as negative or positive depending on percentage of cells showing immunopositivity (<5% cells-negative, 5-100% cells - positive). Ki-67 score was quantified depending on the percentage of positive cells [6] Negative: <5% of Ki-67 positive cells, Grade 1: 5-25% of Ki-67 positive cells, Grade 2: 26-50% of Ki-67 positive cells, Grade 3: >50% of Ki-67 positive cells.

Data Management & Statistical Analysis: All the collected data was documented in an excel sheet and SPSS version 22 was used for statistical analysis.

The expression of Ki-67 and of HPV 16 in premalignant and malignant lesions of cervix was compared to that of controls by using Fisher exact test.

The association between the expression of Ki-67 and HPV 16 was calculated in premalignant and malignant cervical lesions by Chi Square test.

## Results

The study included total 50 cases and out of which 9 cases were premalignant and 41 cases were malignant. 50 controls were also included in the study. The mean age of total cases was 51.5 ± 12.3 years with range of 26-70 years and maximum number of cases was in the age group of 41-50 years (28% of total cases). The mean age of cases with premalignant lesions was 40 ± 10.83 years and with malignant lesions was 53.9 ± 11.28 years. Table 1 shows the various socio-demographic and reproductive characteristics of the cases and controls in the study. It showed that there was significantly higher rate of illiteracy, smoking and low socio-economic status in cases in comparison to controls. Table 2 shows the clinical complaints of the cases showing that vaginal discharge was the most common complaint. Table 3 shows the immunohistochemical expression of HPV 16 and Ki-67in cases and controls.It shows that Ki-67expression was negative in all the controls while 90% of cases showed positivity for Ki-67. Table 4 shows the immunoexpression of Ki-67 and HPV 16 in different histopathological types of premalignant and malignant lesions (Figure 1). There was no statistically significant difference in HPV 16 positivity between LSIL and HSIL and between HSIL and invasive carcinoma. However, with regard to Ki-67 expression, maximum number of premalignant cases (66.7%) showed grade 1 expression for Ki-67 and maximum number of malignant cases (68.3%) showed grade 3 expression for Ki-67. Grade 3 Ki-67 expression was seen in none of the premalignant cases. The malignant lesions of cervix show a significantly higher grade of Ki-67 expression in comparison to premalignant lesions (p value< 0.001).

**Table-1: Socio-demographic and reproductive characteristics of the cases and controls.**

| Characteristics | Cases Number (Percentage of total cases) | Controls Number (Percentage of total controls) | p value |
|---|---|---|---|
| Education Status<br>Illiterate | <br>35 (70%) | <br>22 (44%) | <0.05 |
| Smoking Status<br>Active smokers<br>Passive smokers<br>Non smokers | <br>24 (48%)<br>38 (76%)<br>12 (24%) | <br>13 (26%)<br>20 (40%)<br>30(60%) | <0.001 |
| Socioeconomic status<br>Low socioeconomic<br>Middle socioeconomic | <br>42(84%)<br>8 (16%) | <br>28 (56%)<br>22 (44%) | <0.001 |
| Age of first sexual contact<br>Years (Mean±SD) | <br>18.76±2.25 | <br>20.7±2.12 | <0.001 |
| Age of first conception<br>Years (Mean± SD) | <br>20.86±2.25 | <br>22.5±2.27 | <0.05 |
| Parity<br>Multiparous (Parity>=5) | <br>32(64%) | <br>6(12%) | <0.001 |
| Contraceptive Method used<br>Nil<br>Oral pills, barrier method, Cu T | <br>34 (68%)<br>16 (32%) | <br>23 (46%)<br>27 (54%) | <0.05 |
| Dietary habits<br>Vegetarian<br>Mixed (Vegetarian and non vegetarian) | <br>31 (62%)<br>19 (38%) | <br>25 (50%)<br>25 (50%) | 0.3 |

**Table-2 : Clinical Complaints of the cases.**

| Clinical complaints | Number of cases | Percentage (%) |
|---|---|---|
| Vaginal Discharge | 34 | 68 |
| Bleeding Per Vaginum | 32 | 64 |
| Pain Abdomen | 17 | 34 |
| Heaviness in Vagina | 2 | 4 |
| Itching | 2 | 4 |
| Burning Micturation | 1 | 2 |

**Table-3: Immunohistochemical expression of HPV 16 and Ki-67in cases and controls.**

| | HPV 16 Positive | HPV 16 Negative | Ki-67 Positive | Ki-67 Negative |
|---|---|---|---|---|
| Cases | 39 (78%) | 11 (22%) | 45 (90%) | 5 (10%) |
| Controls | 2 (4%) | 48 (96%) | 0 (0%) | 50 (100%) |

**Table-4: Immunoexpression of HPV 16 and Ki-67 in cervical premalignant and malignant lesions.**

| Histopathological Diagnosis | Number of Cases | HPV 16 Positive | HPV 16 Negative | Ki-67 Positive | Ki-67 Negative |
|---|---|---|---|---|---|
| LSIL | 7 | 7 | 0 | 4 | 3 |
| HSIL | 2 | 2 | 0 | 2 | 0 |
| WD SCC | 1 | 1 | 0 | 1 | 0 |
| MD SCC | 21 | 17 | 4 | 20 | 1 |
| PD SCC | 5 | 4 | 1 | 5 | 0 |
| Basaloid SCC | 5 | 1 | 4 | 4 | 1 |
| MDSCC with Sarcomatoid change | 1 | 1 | 0 | 1 | 0 |
| MDSCC papillary variant | 2 | 2 | 0 | 2 | 0 |
| Adenocarcinoma | 5 | 3 | 2 | 5 | 0 |
| Small Cell Carcinoma | 1 | 1 | 0 | 1 | 0 |
| Total | 50 | 39 | 11 | 45 | 5 |

LSIL, Low Grade Squamous Intraepithelial Lesion; HSIL, High Grade Squamous Intraepithelial Lesion; WD SCC, Well Differentiated Squamous Cell Carcinoma; MD SCC, Moderately Differentiated Squamous Cell Carcinoma; PD SCC, Poorly Differentiated Squamous Cell Carcinoma; SCC, Squamous Cell Carcinoma

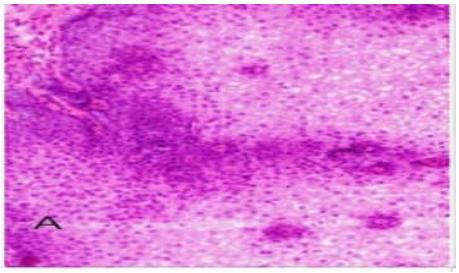
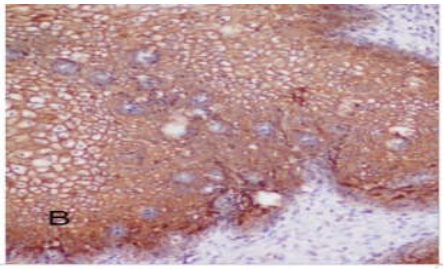
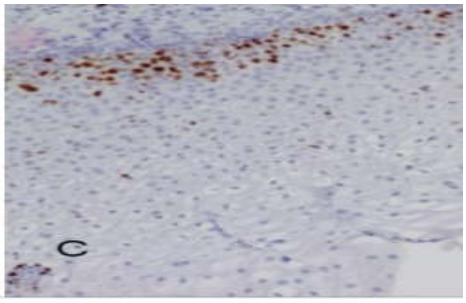


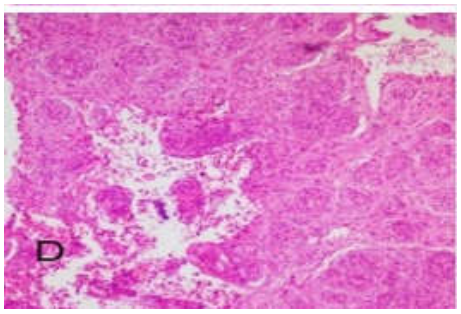

**Figure 1: A) Histopathological section showing HSIL (HE; x10),**



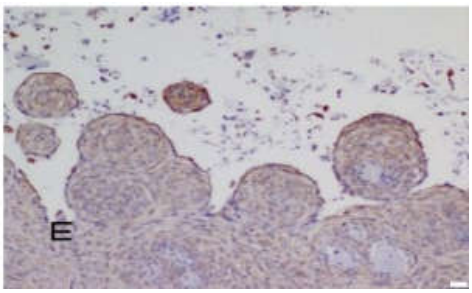

**B) Section showing positive immunohistochemical expression of HPV in HSIL (HPV; x10),**



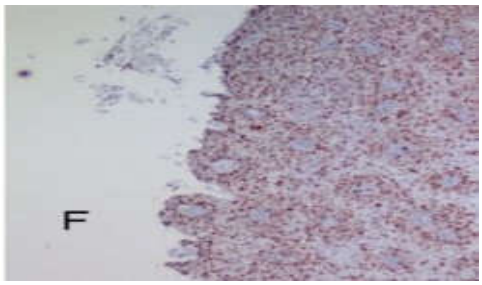

**C) Section showing grade 1+ (positive) immunohistochemical expression of Ki-67 in HSIL (Ki-67; x10)**




**D) Histopathological section showing moderately differentiated squamous cell carcinoma (HE; x4)**




**E) Section showing positive immunohistochemical expression of HPV in moderately differentiated squamous cell carcinoma (HPV; x10),**




**F) Section showing grade 3+ (positive) immunohistochemical expression of Ki-67in moderately differentiated squamous cell carcinoma (Ki-67; x4)**

## Discussion

Cervical cancer ranks as the seventh most common cancer and the fourth most common cancer in females.[1] In the present study, the mean age of cases of malignant cervical lesions was 53.9 years (SD±11.28) while for premalignant lesions was 40 years (SD±10.83). This study is in concordance with a previous study from India which has observed the mean age of incidence of cervical pre malignant and malignant lesions to be 39 years and 49 years with an age range of 31-40 years and 41-60 years respectively [7].

It was observed in our study that premalignant and malignant cervical lesions were associated with significantly higher rate of illiteracy (70%), low socio-economic status (84%) and smoking (76%) in comparison to control. This is usually related to less awareness about causes of cervical cancer and its prevention. In addition, poor personal hygiene and genitourinary infections may also be contributing factor to it. In our study we found that smoking habit (including both active and passive) was more commonly observed in cases than in controls (p value < 0.001). Rouraet al have also observed that smoking status, duration and intensity contributes to a two times increased threat of developing in situ and invasive cervical cancer [8].

In addition, age of first sexual contact, first conception and high parity are also suggested to be important risk factor for cervical malignancy. Previous studies have also found them to be important and self-sufficient risk factors for cervical cancer [9,10]. The significantly increased positivity of HPV 16 expression in cases

(including both pre malignant and malignant) supports the fact that HPV 16 is associated with cervical cancers in this hilly state of India also. The present study also showed that HPV 16 is uniformly present in cervical premalignant and malignant lesions and there is no difference in its expression with severity of the lesion. This is in concordance with previous study by Zouheiret al who have also observed that there is no significant difference in HPV detection rate between HSIL (high grade squamous intraepithelial lesion) and LSIL (low grade squamous intraepithelial lesion) or between HSIL and cervical cancer[11]. In the present study, it was found that maximum number of cases (90%) were positive for Ki-67, amongst which 56% showed grade 3 positivity while all controls (100%) were negative for Ki-67 expression.

It shows that Ki-67 grading was significantly more in cases than in controls (p value < 0.001). Amongst the premalignant cases, maximum number (66.7%) showed grade 1 Ki-67 expression while maximum malignant cases showed grade 3 positivity (68.3%). This shows that grading of Ki-67 increases with the severity of the cervical lesions and may be used as an important immunohistochemical marker to determine the severity of lesion. An interesting finding that was observed in the present study was that 87.2% of the HPV 16 positive cases were also positive for Ki-67 expression which was statistically significantly higher in comparison to controls. However, this association of HPV 16 and Ki-67 did not depend on the severity of the cervical lesions.

This possible reason may be due to equivocal positivity of HPV 16 in premalignant and malignant cervical lesions. In addition, the number of cases included in the premalignant and malignant lesions was also less. Further, an important observation in our study was that 81.4% of total HPV 16 negative individuals (including cases and controls) were also negative for Ki-67 and 82.9% of HPV 16 positive individuals were also positive for Ki-67.

This indicates that HPV16 infection is associated with proliferation of cervical epithelial cells which is indicated by Ki-67 proliferative marker expression and thus this interaction plays an important role in carcinogenesis of cervical cancer.

However an important limitation of the present study was that lesser number of cases were including in the study and therefore larger studies should be done to clearly establish the role of Ki-67as a biomarker in the routine diagnostic work up of cervical neoplastic lesions and its association with HPV 16.

## Conclusion

The study concludes that low socioeconomic status, low education status, smoking (either active or passive), early age of sexual contact, early age of first conception and high parity were associated with increased risk of cervical premalignant and malignant lesions in this region of India.

HPV 16 positivity is associated with positive expression of Ki-67 and thus Ki-67 plays an important role in carcinogenesis of cervical cancer in association of HPV16.

The increase of immuno-positivity of Ki-67 with the severity of lesions suggests its use in stratification of premalignant and malignant lesions of cervix.

**Authors' Contributions**

**UA**: Conception of study, acquisition, analysis and interpretation of data, drafting of manuscript

**S.C.:** Design of the study, analysis and interpretation of data, drafting of manuscript

**UB:** Analysis of data, clinical intellectual input

**VS:** Analysis of data and intellectual input

**What this study adds to the existing knowledge?** Ki-67 plays an important role in carcinogenesis of cervical cancer in association with HPV 16. The increase of immunopositivity of Ki-67 with the severity of lesions suggests its use in stratification of pre malignant and malignant lesions of cervix.

**Findings**: Nil; **Conflict of Interest**: None initiated
**Permission from IRB**: Yes

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