



Human Papilloma Virus 16 and 18 Association in Cervical Intraepithelial Lesions and Cervical Cancers by *In Situ* Hybridization

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ABSTRACT

Objective: To correlate the association of high risk Human Papilloma Virus (HPV) 16, 18 in cervical intraepithelial lesions and cervical cancers by in-situ hybridization (ISH) technique. **Study Group:** Cervical biopsy and hysterectomy specimen of 78 young and adult women, attending Hi-Tech Medical College and Hospital, Bhubaneswar, who were clinically or cytologically suspected of cervical intraepithelial lesion or cervical cancer were taken as source of target viral DNA. **Material:** Formalin 10% as fixative H & E stain as routine staining agent In-situ hybridization kit for HPV 16 and 18 DNA. **Method:** After following standard protocol for surgical grossing, HPV 16, 18 In-situ hybridization kit was used on paraffin embedded tissue sections. **Results:** The percentage of positive cases was highest in cervical cancer patients followed by cervical intraepithelial lesions, high grade, and low grade. **Conclusion:** This study has been carried out for the first in our state and our results show high degree of positivity of HPV 16/18 in females with cervical intraepithelial lesions and cervical cancers attending our tertiary care hospital.

Keywords: HPV 16/18, in-situ hybridization, cervical intraepithelial lesion, cervical cancer

INTRODUCTION

Cervical cancer, mainly caused by Human Papilloma Virus (HPV) infection, is the leading cancer in Indian women. India has a population of approximately 365.71 million women above 15 years of age, who are at risk of developing cervical cancer [1]. The current estimates indicate approximately 132,000 new cases diagnosed and 74,000 deaths annually in India, accounting to nearly 1/3rd of the global cervical cancer deaths [1]. Most cases of cervix cancer are preventable. Cervical Papanicolaou smear and Human Papilloma Virus testing are now recommended in the routine screening programmes. The present study hence has been taken up to observe the scenario of cervical intraepithelial lesions and cervical cancer by assessing the presence of high risk HPV 16 and 18 by *in situ* hybridization technique for the first time in Odisha in a tertiary health care set up.

HPV is the most common viral infection of the reproductive tract [1]. The peak time for acquiring infection for both women and men is shortly after becoming sexually active. HPV is sexually transmitted; skin-to-skin genital contact is a well-recognized mode of transmission [1].

There are four major steps in cervical cancer development [2]:

1. Infection of metaplastic epithelium at the cervical transformation zone.
2. Viral persistence.
3. Progression of persistently infected epithelium to cervical pre-cancer.
4. Invasion through the basement membrane of the epithelium.

MATERIALS AND METHODS

The cross-sectional study was carried over a period of 2 years involving 78 female patients from 1st August 2014 to 31st September 2016 in the PG department of Pathology in collaboration of Department of Obstetrics and Gynaecology of Hi-Tech Medical College and Hospital, Bhubaneswar. The study was approved by Institutional Ethics Committee written consent was taken from the patients.

All female patients belonging to young or adult age, who presented with cervical intraepithelial lesions or cervical cancers diagnosed either clinically or cytologically and admitted to Department of Obstetrics and Gynaecology in Hi-Tech Medical College and Hospital, Bhubaneswar between 1st August 2014 to 31st September 2016 were included in the study.

All female patients with normal cytological cervical Pap smears and histological diagnosis of chronic cervicitis are excluded from the study. Also, patients without adequate history, inadequate sample, unfixed sample, mutilated specimens and patients who did not give consent for the surgery are excluded.

After receiving the sample, it was examined macroscopically and the gross morphology was recorded. The specimen was kept in 10% formalin for 24 h. Tissue section of 5 mm was taken from the appropriate representative sites from the hysterectomy specimen. Biopsy specimen was embedded in toto.

The ZytoFast HPV-Typing ISH Kit of ZytoVision was used for *in situ* hybridization. The digoxigenin-labelled HPV specific oligonucleotides are used as probes. The duplex formation is indirectly detected using an enzyme conjugated antibody targeting the tag: the enzymatic reaction of a chromogenic substrate leads to the formation of a colour precipitate that is visualised by light microscopy. The oligonucleotides target sequences which code for the proteins E6, E7 and L1 are detected by the probe. The chromogen used was BM purple which imparted purple blue to purple pink colour to the precipitates. HeLa cells were used as positive controls for the study.

Statistical analysis of the results was obtained and interpreted in form of graphs and charts.

RESULTS

The present study analysed 78 cases of young and adult females who presented to Department of Gynaecology and Obstetrics in Hi-Tech Medical College and Hospital over a length of two years, i.e. 1st August 2014 to 31st September 2016.

The youngest patient is 23 years of age and the eldest is 78 years. Maximum patients belonged to 41-50 years of age representing 51.28%. There are 21.79% patients belonging to 51-60 years age group making it the second common group. The distribution remains subtle in the other age groups.

The major complains are post-menopausal bleeding (25.64%), discharge per vagina (24.35%) and post coital bleeding (20.51%). Few females also presented with complaints of pain in lower abdomen (19.23%) and pruritus vulva (8%).

Out of 78 cases, 61 cases (78.20%) showed positive signals for the HPV 16/18 DNA. Seventeen cases (21.79%) were negative for HPV 16/18 DNA expression.

Out of the 78 cases, maximum number of positive cases is seen in squamous cell carcinoma (SCC) which constituted 45 out of 46 cases (57.69%). In low grade, squamous intraepithelial lesion (LSIL) (8.97%) 7 cases are positive out of 22 cases and in high grade squamous intraepithelial lesion (HSIL) (8.97%), 7 expressed positive out of 8 cases. Each of the single case of transitional cell carcinoma (TCC) (1.28%) and neuroendocrine carcinoma (NC) (1.28%) are also positive for HPV 16/18. There is statistical significant association between the histopathological cases and HPV 16/18 DNA expression. These observations are elaborated in Figure 1A and 1B.

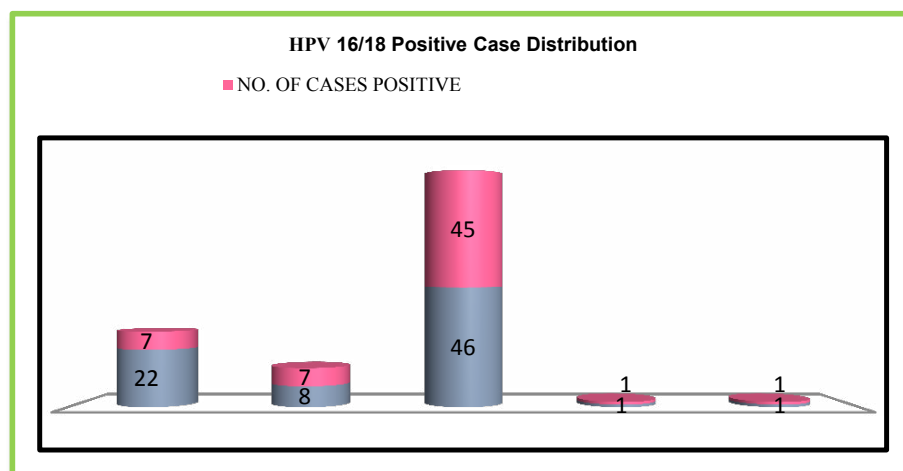


Figure 1A Distribution of HPV 16/18 DNA positive cases in the individual histopathological types (n=78)

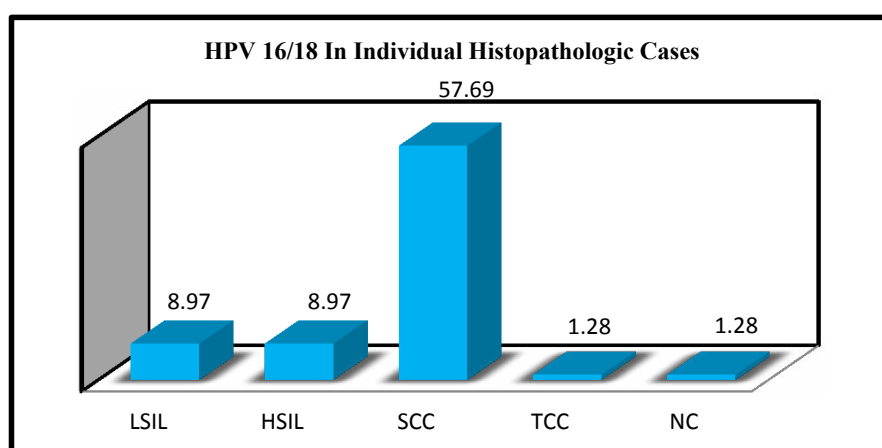


Figure 1B Bar graph representing percentage of HPV 16/18 DNA positivity in the various histopathological types (n=78)

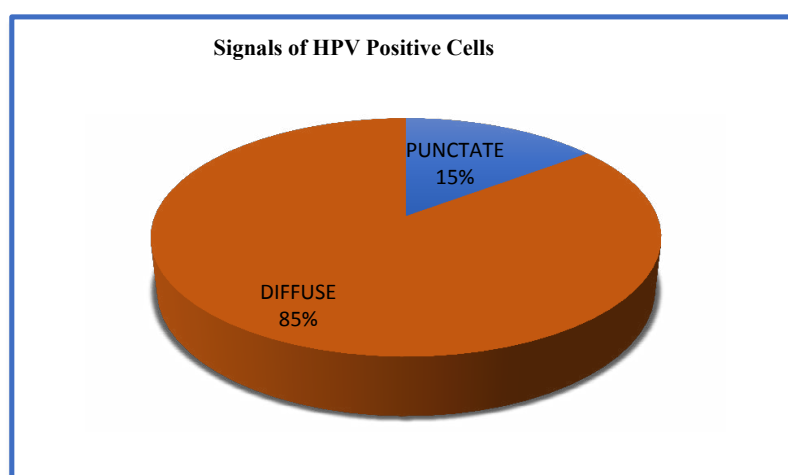


Figure 2 Pie chart representing the percentage distribution of signal pattern in HPV 16/18 DNA positive cells

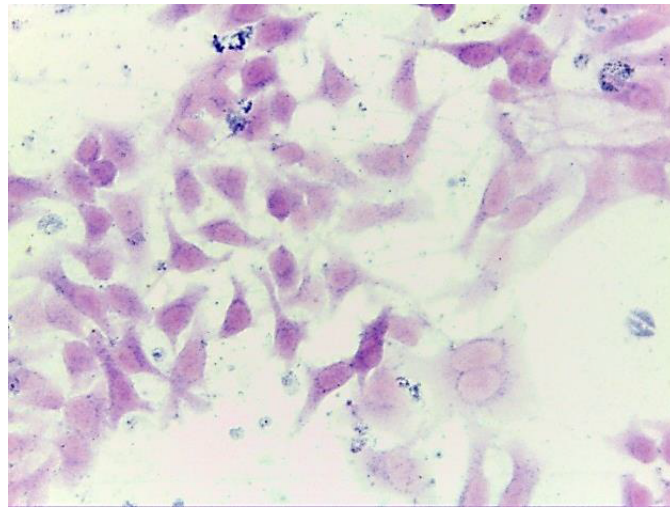


Figure 3 HeLa cells showing HPV 16/18 positivity by ISH

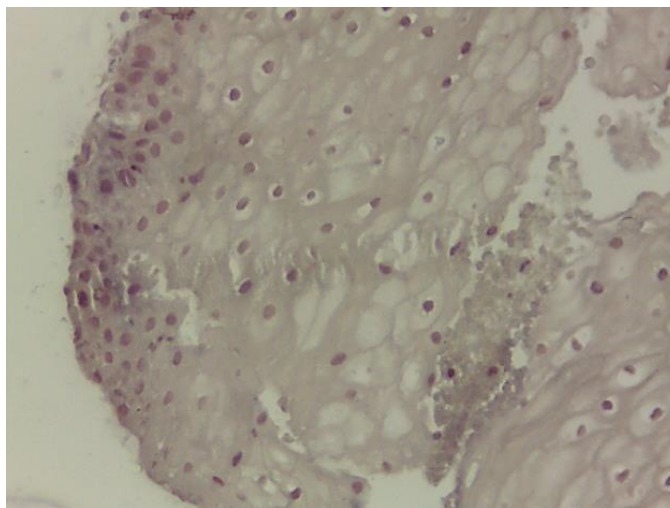


Figure 4 LSIL HPV 16/18 positivity by ISH

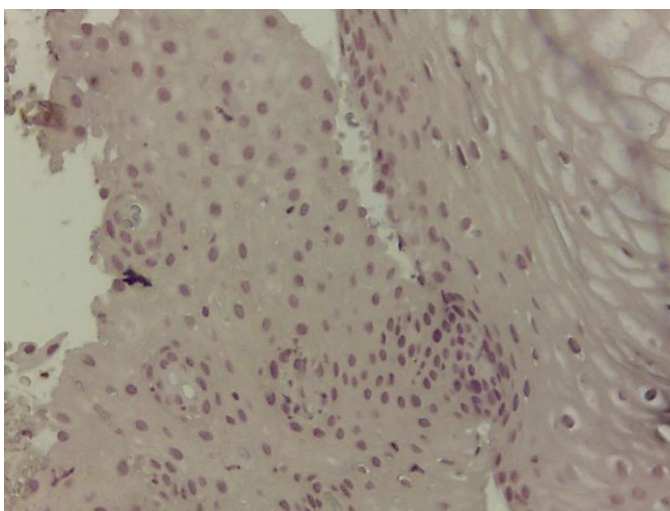


Figure 5 HSIL showing HPV 16/18 positivity by ISH

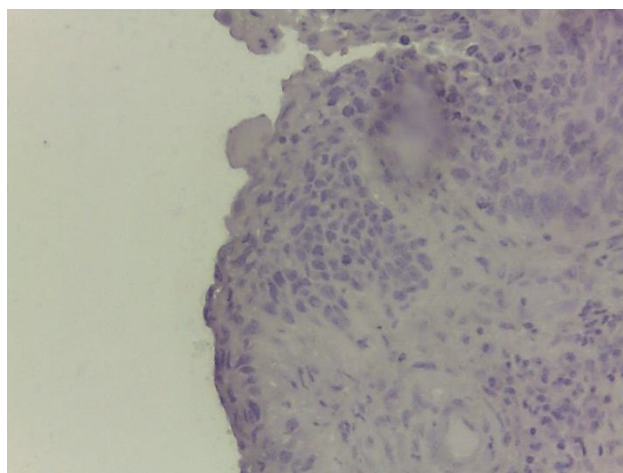


Figure 6 SCC showing HPV 16/18 positivity by ISH

Study of the signal patterns of HPV positive cells showed two categories (Figure 2). One pattern showed granular/punctate signals/dot like signals in the nucleus of the positive cells constituted (Figures 3-6). The other pattern shows diffuse nuclear staining. There are 7 cases of LSIL and 2 cases of HSIL showing 9% punctate positive signals. The diffuse staining of the nucleus was observed in 5 cases of HSIL and 45 cases of SCC making 85.24% of the 61 positive cases.

DISCUSSION

Cancer of the cervix is the most studied cancer and its precursor lesions have undergone extensive investigation. Probably, cervical cancer is the only gynaecological cancer that satisfies the well-recognized WHO criteria for implementation of a screening program. Hence, extensive studies are going through worldwide. The current study included 78 cases of cervical intraepithelial lesions and cervical cancers which came to our institution during the period of August 2014 to September 2016.

Table 1 Comparative analysis of HPV16/18 positivity

Study	Place of Study	Positive Cases/Total cases	Percentage (%)
Gupta, et al. [3]	USA	50/70	71
Zhang, et al. [4]	China	4/23	17.39
Guimarães, et al. [5]	Brazil	6/30	20
Menon, et al. [6]	India	55/72	76.4
Choudhury et al. [7]	India	24/50	48
Present Study, 2016	India	61/78	78.2

The present study has 61 cases positive out of 78 cases accounting for 78.20%. Gupta, et al. [3] had 71% cases positive, Zhang, et al. [4] had 17.39% cases positive, Guimaraes, et al. [5] had 20% of positive cases, Menon et al. [6] had 76.40% and Choudhury, et al. [7] had 48% cases positive (Table 1).

No consistent pattern emerged in the studies both in developed and developing countries. The reasons for these variations could be many including criteria employed for diagnosis, the quality checks used, the use of stringent methods during the staining procedure, intrinsic differences in the population studied including the prevalence of risk factors and the number of cases studied.

Table 2 Comparative analysis of HPV 16/18 positivity in cervical intraepithelial lesions with various studies

Study	Place of Study	LSIL		HSIL	
		Cases	%	Cases	%
Amortegui, et al. [8]	Pennsylvania	102/157	65	20/20	100
Kyo, et al. [9]	Japan	04/7	57.14	06/9	66.67
Pich, et al. [10]	Italy	3/15	20	07/9	77.8

Leu, et al. [11]	Taiwan	0/8	0	05/9	55.56
Present Study, 2016	India	7/22	31.81	07/8	87.5

The present study separately compared its incidence and HPV positivity of precursor lesions and malignant lesions with various studies around the world. In the present study, LSIL constitutes 31.81% and HSIL constitutes 87.50% positive cases (Table 2).

This variation in positivity from all the studies belonging to south-east Asian region implies infection by HPV is not the only parameter to develop carcinoma. The association of risk factors like, age at first coitus, multiple partners, parity, smoking, personal hygiene etc. play a significant role not only in de novo cervix carcinoma but also in progression of the intraepithelial lesions to malignancy (Table 3).

Table 3 Comparative analysis of HPV positivity in cervical squamous cell carcinoma with various studies

Study	Place of Study	SCC	
		Cases	%
Leu, et al. [11]	Taiwan	46/46	97.82
Siritantikorn, et al. [12]	Bangkok	5/18	29
Kyo, et al. [9]	Japan	06/6	100
Lu, et al. [13]	China	23/48	47.92
Nimmanahaeminda, et al. [14]	Thailand	31/52	59.61
Menon, et al. [6]	India	20/68	29.4
Present Study, 2016	India	15/22	68.18

CONCLUSION

The present study focused on HPV detection by *in situ* hybridization in correlation with histologic findings. The study shows that when used together and evaluated in conjunction with histologic sections, *in situ* hybridization is a useful tool for ancillary molecular testing of HPV infection in cervical lesions. Because the integration of oncogenic HPV into the human genome is a critical step for pathogenesis of cervical cancer, the signal pattern of HPV, which may suggest viral integration status, can be used as a helpful marker for predicting precancerous lesion progression. Despite these advantages, the *in-situ* hybridization assay has a low sensitivity, which is a major concern for pathologists who use *in situ* hybridization to detect HPV in tissue.

In conclusion, a high prevalence of high risk HPV DNA is evident in population of Odisha from the present study. This approach may lead to more appropriate screening strategies after the implementation of HPV vaccination and may be needed to fully evaluate the prevalence of HPV in cervical tissues. An investigation of a larger population using HPV-ISH analysis will be necessary to measure its usefulness and determine whether prognostic differences related to HPV physical status exist.

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