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ORIGINAL PAPER

HPV Subtype 16 and 18 Distribution Among Young Sexually Active Women with Vaginal Discharge or Cervical Lesion

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ABSTRACT

Objective: To study the prevalence of HPV infection and common subtypes 16 and 18 in young sexually active reproductive age group females with vaginal discharge or cervical lesion and to know the other determinants of HPV infection. Methods: Total 110 sexually active reproductive age group female with vaginal discharge or cervical lesion were included in the study and dry paper smear of cervical scraping were collected for HPV DNA testing by PCR. Results: The prevalence of HPV is 9.09%, HPV 16 is 3.64%, HPV 18 is 0%. Conclusion: The present study also shows significant association of HPV infection with younger age, early initiation of sexual activity, associated symptoms like white discharge or irregular vaginal bleeding, H/O other sexually transmitted infections and abnormal pap smear report.

***Keywords**: Human papilloma virus (HPV), deoxyribonucleic acid (DNA), polymerase chain reaction (PCR)*

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INTRODUCTION

Cervical cancer affects approximately 1.4 million women worldwide and claims an estimated 239000 lives each year. Over 99% of cervical cancer cases result from genital infection with human papillomavirus (HPV). The disease represents a major health inequity, as 80% of those with cervical cancer live in developing countries. An estimated 660 million people worldwide have HPV genital infections, the most common viral infection of the reproductive tract. The peak incidence of HPV infection occurs in adolescents and young adults under 25 years of age.[1]

HPVs are non-enveloped, double-stranded DNA viruses[2] in the papillomaviridae family with circular genome enclosed in a capsid shell comprising major (L1) and minor (L2) structural proteins which infect epithelial cells of the skin or mucosa. HPV genomes also encodes several 'early' genes (E1-E7) that direct viral transcription and replication and interact with the human genome.[3]

High-risk HPV types have the genes E6 and E7, which are associated with cell immortalization and transformation related to carcinogenesis. The HPV types are most strongly associated with invasive cervical cancer and other HPV-related cancers are 16 and 18. For invasive cervical cancers, the next most common types include 31, 33 and 45. The dominant oncogenic type in all regions is HPV16. Cervical cancer is the most common HPV-related malignancy worldwide and is the second most common type of cancer among women. Risk factors for transmission and acquisition of HPV infection include multiple sex partners, lack of condom use, smoking, and co-infection with other sexually transmitted infections

(STIs), including HIV, a male partner with multiple sex partners. Longitudinal and cross-sectional studies demonstrate that the most consistent predictor of HPV infection in women is the number of lifetime or recent sex partner.

Carcinoma of the uterine cervix is the second most common cancer in the districts of Dibrugarh, Kamrup Urban and third in Silchar Town with AAR of 11.8%, 13.1% and 13.9%.(ICMR, 2003-2004) Despite the high incidence of cervical cancer reported from Assam, a state in north east India, population based or hospital based studies on the HPV prevalence and genotypic distribution are very few from this region. A PCR based study detected 24.0% HPV prevalence among suspected cervical cancer patients attending Silchar Medical College at Silchar, southern Assam.[4] Incidences and mortality of cervical cancer is highest in countries where effective screening, diagnosis and treatment are absent or limited. It is thus expected that carrying out studies on HPV in this part of the country would throw some light on prevalence of HPV 16 and 18 among sexually active reproductive age group female with white discharge or cervical lesion. It will help in adopting measures for prevention as well as improve outcome in cases of cervical cancer.

MATERIAL AND METHODS

A cross sectional, observational study was carried out in the gynaecology OPD, Department of Obstetrics and Gynaecology, Gauhati Medical College and Hospital, Guwahati. A total of 110 women in reproductive age group who are sexually active with cervical lesion and vaginal discharge were studied. Patient particulars were noted, detail history including sexual behaviour of the patient and partner, history of STI, general and systemic examination were done and a total of 110 dry paper smear of cervical scraping were collected.

The research topic has already been approved by the Institutional Ethical Committee. The study was conducted from 1st June 2012 to 31st May 2013. Cervical scraping from the posterior vaginal pool was smeared onto Whatman 3 MM filter paper, and stored individually at room temperature in air-locked zip-lock bags after labelling for HPV DNA testing by PCR. The dry paper smears were carried to the Regional Medical Research Centre (RMRC) for North East India situated at Dibrugarh, for detection of HPV DNA by PCR. The HPV DNA PCR was carried out according to the method described by Usha Sharma et al.[5]

All data were analyzed by applying **Pearson's Chi-Square test** ($\div^2$) of significance. A 'p' value of more than 0.05 was considered to have no significant variation, p value less than 0.05 was considered significant and 'p' value less than 0.001 was considered to have highly significant variation.

OBSERVATION AND RESULT

1. **Prevalence of HPV**: Prevalence of HPV in 110 cases is shown in **Table 1**.

Table 1 Results of HPV infection

Total test done	HPV +ve (9.09%)	HPV –ve (90.91%)
110	10	100
	HPV 16=4(3.64%)	
	HPV18=0(0 %)	
	HPV other=6 (5.45%)	

2. Age: The association of HPV status with age of 110 cases are narrated in **Table 2.** It has been found that there is a significant association between the age factor and HPV status ($p<0.01$), i.e., younger the age group more prevalence of HPV.

Table 2 Association of HPV status with age

Age (years)	HPV +ve	HPV -ve	Total	d.f.	x^2-value
<25 years	0	6	6	5	16.693* ($p<0.01$)
25-29yrs	0	29	29		
30-34yrs	4	24	28		
35-39 yrs	3	25	28		
40-44 yrs	1	15	16		
45 yrs & above	2	1	3		
Total	**10**	**100**	**110**		

3. No of pregnancy: There is no significant association between number of pregnancy and HPV status ($p>0.05$) as shown in **Table 3.**

Table 3 Association of HPV status with the no. of pregnancy

No.of pregnancy	HPV +ve	HPV –ve	Total	d.f.	x^2-value
0	0	3	3	4	2.355 $p>0.05$
1	0	15	15		
2	3	30	33		
3	4	29	33		
4 & above	3	23	26		
Total	10	100	110		

4. Socio-economic status, educational attainment and religion: No significant association was found between socioeconomic status, educational attainment and religion with HPV infection.

5. Age at first sexual exposure: Significant association was found between the incidence of HPV and the age of first sexual exposure as shown in **Table 4**.

Table 4 Distribution of cases according to their age at first sexual exposure

Age at first sexual exposure	HPV+ve	HPV-ve	Total	d.f.	x^2-value
≤20	9	79	88	2	6.188* (p<0.05)
21-25	0	20	20		
26-30	1	1	2		
Total	10	100	110		

6. Duration of marriage and history of contraception: No significant association was found between the incidence of HPV and the duration of marriage (p>0.05) and history of contraception.

7. History of vaginal discharge: Significant association was found between presence of white discharge and HPV status as shown in **Table 5**.

Table 5 Association of HPV status with the history of vaginal discharge

Vaginal discharge	HPV +ve	HPV -ve	Total	d.f.	x^2-value
Yes	7	91	98	1	4.125* (p<0.05)
No	3	9	12		
Total	10	100	110		

8. History of vaginal bleeding: A highly significant association has been found between vaginal bleeding and HPV status as shown in **Table 6**.

Table 6 Association of HPV status with the history of vaginal bleeding

Vaginal bleeding	HPV +ve	HPV -ve	Total	d.f.	x^2-value
Yes	5	92	97	1	15.388** (p<0.01)
No	5	8	13		
Total	10	100	110		

9. PAP smear Test: A highly significant association has been found between the PAP smear test and HPV status (with p<0.01).

Table 7 Association of HPV status with Pap smear test category

Pap smear	HPV +ve	HPV -ve	Total	d.f.	x^2-value
HSIL/ECA	9	7	16	1	50.385** (p<0.01)
NILM	1	93	94		
Total	10	100	110		

10. History of STI: A highly significant association has been found between the H/O STI and incidence of HPV (p<0.01).

Table 8 Association of HPV status with the history of STI

History of any STIs	HPV +ve	HPV -ve	Total	d.f	x^2-value
yes	6	9	15	1	20.078** (p<0.01)
No	4	91	95		
Total	10	100	110		

11. HPV and multiple sex partners: No significant association has been found between the H/O multiple sex partner and prevalence of HPV (p>0.05).

12. History of having male partner with multiple sex partners: No significant association has been found between the H/O having male partner with multiple sex partner and prevalence of HPV (p>0.05).

DISCUSSION

The present study showed a lower prevalence of HPV compared with the study done by Laikangbam P et al[6] in Sikkim and Kulkarni SS et al[7] in Karnataka, but similar results were found in the study done by Srivastava S et al[8] in Uttar Pradesh and in Eastern India by Dutta et al[9]. In Manipur, a neighbouring state in Assam, lower prevalence of HPV has been observed. Present study has almost similar findings with that of lower Assam by Usha Sarma et al.[5]

The age group of the present study is similar to the study of Kataja et al 1993[10], Gradiolone et al[11] and Monk J Bradley et al.[12]

There was no significant association between HPV status

and number of pregnancy. Dutta et al[9] found significant association between HPV infection and women with parity e"4. No significant association found between socioeconomic status with HPV infection. Similar findings were reported by Cherian Varghese[13] and Lar Munoz et al.[14] No significant association found between religions with HPV infection. Similar findings were reported by Cherian Varghese[13] and Laikangbam et al.[6]

In the present study significant association was found between the incidence of HPV and the age of first sexual exposure and these finding was supported by Kenny et al[14] and Jessica A. Kahn et al.[15] The findings of vaginal discharge was supported by the findings of Paul K S Chan.[16] A highly significant association has been found between the PAP smear test and HPV status which was supported by the findings of Cherian Varghese[13] and Frega A et al.[17] A highly significant association has been found between the history of sexually transmitted infection and incidence of HPV which has been supported by **Petroula Stamataki** et al[18] and Brinton et al.[19]

No significant association has been found between the history of multiple sex partners and prevalence of HPV ($p>0.05$). Munoz et al.[14] reported 4 fold increase of HPV infection in women who had 6 or more partner. In conservative societies like ours promiscuity is always underreported; this may be the reason why no significant association has been found for this factor in the present study.

No significant association has been found between the history of having male partner with multiple sex partner and prevalence of HPV ($p>0.05$). However Kenny[20] reported increase in risk of HPV infection in women with male partners having multiple sexual partners.

CONCLUSION

High-risk HPV types 16 and 18 are responsible for the great majority of HPV-related malignant diseases and screening of women for this etiological agent could be a potentially applicable measure in early detection of the disease. Mortality rates are highest in low-income countries, where most disease is detected at late stage because there is little or no access to screening and effective treatment. The present study shows a prevalence of 9.09% of HPV infection among sexually active reproductive age group female with white discharge or cervical lesion. The present study also shows significant association of HPV infection with younger age, early initiation of sexual activity, associated symptoms like white discharge or irregular vaginal bleeding, H/O other sexually transmitted infections and abnormal pap smear report. Such information is important for evaluating importance of the early screening and use of anti-HPV vaccines as a public measure in controlling the burden of cervical cancer.

Conflict of interest: None declared.

Ethical clearance: Done.

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