

Prevalence Of HPV In Hospital Set Up And Its Correlation With CIN

A Khanna, A Matah, M Kumar

Citation

A Khanna, A Matah, M Kumar. *Prevalence Of HPV In Hospital Set Up And Its Correlation With CIN*. The Internet Journal of Gynecology and Obstetrics. 2008 Volume 10 Number 2.

Abstract

One hundred women were studied and the presence of high risk (HR) HPV infection was found in 12% women. On cytological examination, 17% women had normal smear, 49% inflammatory smear, 5% ASLVS, 16% LSIL, 5% HSIL, 4% Koilocytosis. Eight percent women who had HSIL in cervical cytology were HR HPV positive and 50% who had koilocytosis in Pap smear were HPV positive.

All the HPV positive cases (30%) in the LSIL group were less than 30 years suggesting high prevalence of transient infection in this age group. HSIL cases were 30 years or above in age and 80% HPV positive cases in the HSIL group were also above 30 years. After age 30, a positive DNA test correlated well with cervical disease and represented a persistent infection. So screening programs for cervical cancer should use tests for HPV in women 30 years of age or older.

Colposcopy was done in 70% patients. HPV positivity went on increasing with increasing grades of colposcopic cases; 60% HPV positivity was noted with grade III changes and 100% positivity with findings suggestive of malignancy.

The correlation between histopathology (colposcopic directed biopsy) and HPV DNA testing suggested a strong association between HPV positivity with higher grades of CIN. HPV positivity was 33.2% in CIN 2 cases; 75% in CIN 3 and 100% in cases diagnosed as carcinoma in situ.

INTRODUCTION

Globally, cervical cancer is the second most common cancer in women and the third most frequent cause of cancer death. Cancer of cervix is preventable, yet approximately 493,100 new cases and more than 273,000 deaths occur each year among women worldwide. India, which accounts for one sixth of the world's population also bears one fifth of the world's burden of cervical cancer. There is excellent evidence that invasive squamous carcinoma of the uterine cervix develops from abnormal cancerous surface epithelium, borderline lesions or dysplasias. Cervical cancer is the end stage of the continuum of increasingly severe abnormalities in the squamous epithelium.

Identifying the disease in the early stages has driven researchers to focus on the underlying cause of cervical cancer and how it can be detected at the molecular level. There is overwhelming evidence that virtually all cases of cervical cancer are caused by the Human papilloma virus (HPV), making it perhaps the first cancer to be recognized as

virally induced. The HPV Cervical Cancer model has become a paradigm of progress in cancer research and among neoplastic diseases with infectious roots.

There is growing scientific evidence to suggest that the ability to identify the presence of high risk types of HPV is a key factor in combating this disease at the molecular level. Enormous studies have been made in the HPV – Cervical Cancer area. New testing methods for Human Papilloma Virus (HPV), such as the Digene Hybrid Capture 2 HPV DNA Test (hc2 HPV DNA test), present both opportunities and challenges in the diagnosis and management of cervical carcinoma.

MATERIALS AND METHODS

The present work was carried out on 100 women presenting to the Female Outpatient department from July 2004 to June 2006 of University Hospital, Banaras Hindu University, Varanasi, India. The Inclusion Criteria were as follows :

Patients \geq 30 years of age were subjected to primary

screening using hC2 HR HPV DNA, Pap Test and colposcopy when needed.

Patients ≤ 30 years of age with abnormal Pap smear or inflammatory smear with unhealthy cervix were subjected to hC2 HR HPV DNA test and colposcopy.

All women were subjected to the detailed history, clinical examination, high risk HPV DNA detection by Hybrid Capture 2 technique, cervical cytology (Pap smear), colposcopy. HPV DNA was detected in this study by the Digene's hC2 HR HPV DNA Test.

Cervical specimens were collected and transported using the DNA Pap cervical sampler or the HC cervical sampler (consisting of a Cervical Brush and a Specimen Transport Medium) in a specimen transport medium. Excess mucus was removed from cervical os and surrounding ectocervix using a cotton swab.

Brush was inserted 1-1.5 cm into the cervical os until the largest outer bristles of the brush touched the ectocervix. It was rotated 3 full turns in a counterclockwise direction. Brush was removed from the canal. Brush was inserted to the bottom of transport tube. Sampler shaft was then snapped off at score line, leaving the brush end inside the tube. The tube was recapped securely by snapping it in place. Cervical specimens in STM were sent without refrigeration to the testing laboratory (specimens may be held at room temperature for 2 weeks).

HC2 HIGH RISK HPV DNA TEST

The Digene HPV test or hC2 High Risk HPV DNA test was used to screen for the presence or absence of high risk oncogenic HPV types that can cause cervical cancer, providing an objective risk indicator for the development of high grade cervical disease and cancer. The hC2 high risk HPV DNA test using Digene's Hybrid Capture 2 (hC2) technology is a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative detection of thirteen high risk types of human papillomavirus (HPV) DNA in cervical specimens.

STM specimen's RLU / positive control cut off ratio was calculated. In this study, ratio 0 to 0.8 was taken as Negative, Ratio 0.81 to 1.20 was taken as Borderline, Ratio >1.2 was taken as Positive. The ratio at 1.0 indicated HPV load of 5000 copies / ml at a threshold of clinical significance to progression of cervical abnormality.

CERVICAL CYTOLOGY : (PAP SMEAR)

The smear was prepared from the cervical scrapes obtained with the help of wooden (Ayre's) ₂ spatula with cytobrush and smeared on a glass slide and fixed for modified Pap staining. ₃ (Koss). All papanicolaou smears were read by one investigator and were categorized according to Bethesda System for cervical / vaginal cytology diagnosis.

To condense the whole descriptive terminology, the results in this study were grouped into 6 groups as follows. Normal, Koilocytosis, Inflammatory, Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL), Atrophic smear.

COLPOSCOPY

Colposcopy provided an optical method for examining the illuminated cervix, the lining layer of the vagina and lower endocervical canal. It was conducted on all females with unhealthy cervixes and abnormal pap smear. The grading was done as per findings obtained according to the system proposed by Coppersons (1987) ₄. Colposcopic directed biopsy was taken and subjected to histopathological examination.

Disease status was based on the results of histologic evaluation, however, when histopathology was negative or in the absence of histology result, disease status was determined by cytology at the time of colposcopic examination.

OBSERVATIONS

Out of 100 cases, 12% were positive for high risk HR HPV and 88 were negative. The age of the patients ranged from 21-60 years. Out of 100 cases, 48 cases (48%) were between 21 – 30 years; 40 cases (40%) were between 31 – 40 years; 11 cases (11%) between 41 – 50 years and 1 case (1%) was between 51 – 60 years. Seven (14.5%) patients with HR HPV DNA were in the age group 21 – 30 years, 4 (10%) in 31 – 40 years and 1 (9%) in 41 – 50 years. Maximum HR HPV positive patients (58.33%) were in the age group 21 – 30 years and this positivity decreased as the age increased. (Table 1)

Figure 1

Table 1 : Correlation of HR HPV positivity with age. (n = 12)

Age group in years	No. of cases	No. of cases with HR HPV DNA	Percentage
21 – 30	48	7	58.33
31 – 40	40	4	33.33
41 – 50	11	1	8.34
51 – 60	1	0	-
Total	100	12	

On Pap smear cytology , 17 cases (17%) had normal smears; 49 (49%) inflammatory, 5 (5%) ASCUS, 16 (16%) LSIL, 5 (5%) HSIL, 4 (4%) with Koilocytosis and 4 (4%) cases had atrophic smears. Three patients (6.1%) were HR HPV positive in inflammatory smear group, 3 (18.7%) in LSIL group, 4 (80%) in HSIL group, 2 (50%) were positive in Koilocytosis group. (Table 2)

Figure 2

Table 2 : Relation between Cervical cytology and HR HPV positivity.

Cytological class	No. of cases (n = 100)	Percentage	No. of HR HPV positive cases	Percentage
Normal	17	17	-	-
Inflammatory	49	49	3	6.1
ASCUS	5	5	-	-
LSIL	16	16	3	18.7
HSIL	5	5	4	80.0
Koilocytosis	4	4	2	50.0
Atrophic	4	4	-	-

ASCUS : Atypical Squamous Cell of Undetermined significance

LSIL : Low grade Squamous intraepithelial lesion

HSIL : High grade Squamous intraepithelial lesion

Colposcopy was done in 70 (70%) patients (who had abnormal Pap smear or inflammatory smear with unhealthy cervix). It was observed that HPV positivity went on increasing with increasing grades of colposcopic class; 20% HPV positivity was seen with grade II changes, 60% with grade III changes and 100% with findings suggestive of malignancy, even (10%) cases showed features suggestive of HPV infection of colposcopy and 4 (57%) of these were positive for HR HPV DNA. (Table 3)

Figure 3

Table 3 : Prevalence of HR HPV in different colposcopic categories.

Colposcopic finding	No. of cases (n=70)	Percentage	HR HPV DNA positivity	Percentage
Normal	22	31.4	-	-
HPV changes	7	10.0	4	57.1
Grade I changes	20	28.5	1	5.0
Grade II changes	15	21.4	3	20.0
Grade III changes	5	7.1	3	60.0
Suggestive of malignancy	1	1.4	1	100

HR HPV was positive in 1 case (25%) of condyloma, 3 cases (15.7%) of CIN I, 1 case (33.3%) of CIN 2; 3 cases (75%) of CIN 3 and 2 cases (100%) of carcinoma in situ group. (Table 4) The cases which showed lower grade of the disease on cytology were actually of higher grade on histopathology. (Table 5)

Figure 4

Table 4 : Correlation of histopathology (colposcopic directed biopsy) and HR HPV positivity by hc-2 test.

Histopathology (colposcopic directed biopsy)	No. of cases (n= 70)	Percentage	No. of HR HPV positive cases	Percentage
Chronic cervicitis	38	54.2	2	5.2
Condyloma	4	5.7	1	25
CIN 1	19	27.1	3	15.7
CIN 2	3	4.2	1	33.3
CIN 3	4	5.7	3	75.0
Carcinoma in situ	2	2.8	2	100
Total	70	100		

Figure 5

Table 5 : Correlation of histopathology (colposcopic directed biopsy) and cervical cytology. (n=70)

HPE (colposcopy directed biopsy)	Cytological class					
	Inflammatory	ASCUS	Koilocytosis	LSIL	HSIL	Atrophic
Chronic cervicitis (38)	30	4	-	-	-	4
CIN 1 (19)	5	1	1	12	-	-
CIN 2 (3)	-	-	-	3	-	-
CIN 3 (4)	-	-	-	1	3	-
CIS (2)	-	-	-	-	2	-
Condyloma (4)	1	-	3	-	-	-
Total	36	5	4	16	5	4

DISCUSSION

Recent studies have shown that high risk HPV is present in more than 99.7% of cervical carcinomas worldwide. Persistent infection with high risk HPV is necessary for the development and maintenance of high grade CIN and invasive carcinoma.

The prevalence of HPV infection varies in different regions of the world. Using hC2 HR HPV DNA test, twelve percent cases were positive for HR HPV and the remaining 88% were negative for HR HPV. Sankarnarayanan et al⁵ have also reported the prevalence of HR HPV (using hC2 test) as 10.3 percent in West India. Nijhawan et al⁶ have mentioned the prevalence of HPV infection to range from 15-20 percent from Chandigarh. Epidemiological studies conducted by Nobbenhuis et al⁷ have shown that the risk of cervical cancer does not depend on the type of high risk HPV with which a woman is infected. Consequently, for clinical purposes related to cervical cancer, tests that detect infection with any high risk HPV type are sufficient. So, the hybrid capture II system is suitable for mass screening.

As per recommendations of ACOG (2003) cervical screening with cytology plus high risk HPV DNA testing should be done in women aged 30 and above, but as the age of development of cervical cancer is much lower in our country, in the present study the patients with abnormal Pap smear and those with inflammatory smear with very unhealthy cervixes, HR HPV DNA testing was carried out even below 30 years of age. Out of total HR HPV positive patients, maximum (58.33%) patients were in the age 21 – 30 years and this positivity decreased as the age increased. This may reflect clearance of HPV infection by immunological effect. Similar observations have been made by de Villiers et al⁸ who found HPV positivity to be higher in younger age groups. Herrero⁹ also believes that a positive HPV DNA test result is specially prevalent among young sexually active women reaching a peak around 20 – 24 years. Prevalence gradually declines with the age until 40 – 45 years.

When a correlation of cervical cytology was made with HPV positivity it was observed that 80% cases who had HSIL in cervical cytology were positive for HR HPV. The patients who showed koilocytosis in cervical cytology had HPV positivity in 50% cases, and 18.7% cases were positive for HR HPV in cases whose cervical cytology was LSIL. In our study, 6.1% patients with inflammatory smears were HPV positive.

Our findings are in close conformity with the data given by the University of Pittsburg (Infectious Diseases and Microbiology Graduate School of Public Health). According to them, HR HPV is present in more than 95% cases of invasive carcinoma, 79 – 90% cases of HSIL, 50 – 75% cases of LSIL, and in 2-5% of normal smears. Toon et al¹⁰

detected HPV DNA in 15% of inflammatory smears and Law et al¹¹ found HPV DNA in 12% of inflammatory smears.

Colposcopy was done in 70 (70%) patients (who had abnormal Pap smear or inflammatory smear with unhealthy cervix); 10% patients showed HPV changes; 28.5% Grade I changes, 21.4% grade II changes, 7.1% grade III and 1.4% had features suggestive of malignancy. It was observed that HPV positivity went on increasing with increasing grades of colposcopic class; 20% HPV positivity was noted with grade II changes, 60% with grade III and 100% with findings suggestive of malignancy.

When a correlation between histopathology (colposcopic directed biopsy) and HR HPV positivity was done, it was noted that HPV positivity was 25% in cases of condyloma; 15.7% in CIN 1, 33.3% in CIN 2 and it steeply rose to 75% in CIN 3 cases and 100% in cases which were diagnosed as carcinoma in situ. It is evident that there is a strong association of HPV positivity with higher grades of CIN.

According to Schiffman et al¹² also more than 90% of high grade dysplasias and invasive cervical cancers have been associated with HR HPV types. HPV DNA is found in 99.7% of all cervical cancers. Nobbenhuis⁷ also holds the same view.

Some of the higher grades of CIN which were missed on cervical cytological examination were detected by histopathology of the biopsy specimen obtained after colposcopic examination (n=70). So the detection of CIN by hc-2 test was more accurate than by papsmear cytology (considering histopathology as gold standard for its diagnosis).

Kulasingam et al¹³ determined the accuracy of HPV DNA testing for detecting cervical intraepithelial neoplasia (CIN). They found the sensitivity of thin layer Pap for identifying women with CIN 3 or higher was only 61.3%, compared with 88.2% for HPV testing by PCR and 90.8% by signal amplification.

The ICMR annual report 2002-2003 mentions that various studies conducted at ICPO (Institute of Cytology and Preventive Oncology, New Delhi) have shown that HPV screening using hybrid capture technology has 75% sensitivity for detection of CIN 1 lesions and nearly 100% sensitivity for detection of high grade (CIN 2, 3) lesions with a specificity of about 83%. Though the positive predictive value was rather low (6.7%), a very high negative predictive

value of 99.2% makes it an ideal tool for Indian situation where frequent screening, as is being done in western countries, is not possible. So, a potentially attractive option might be “once-in-a lifetime” screening for high risk HPV at 35 years of age.

Also a joint statement recently issued by the World Health Organization and the European Research Organization on Genital Infection and Neoplasia at an International Meeting stated that HPV testing showed 95 – 100% sensitivity for high grade cancer precursors compared with 40 – 85% for traditional cervical cytology (Pap test). In fact, the statement strongly suggested that HPV testing should be adopted as the primary screening method for women over age 30.

CONCLUSION

India is a high risk country for cervical cancer, with a high disease burden. Screening programmes are currently the most effective treatment to prevent cervical cancer. Although pap test has reduced the incidence of cervical cancer, yet thousands continue to die of this disease. The pap smear does not confirm the presence or absence of HPV, which is the primary cause of cervical cancer. The advent of HPV testing has opened the doors for surveillance mechanisms other than routine cytological screening. Finding DNA infected with HR HPV suggests the presence of a precancerous lesion, particularly in women with an abnormal or atypical pap test. These high risk patients should have colposcopy and treatment if necessary.

It is concluded from the present study that HR HPV DNA testing along with smear in women more than 30 years of age is an excellent screening modality. This confirms that a patient with abnormal results is at high risk of developing cervical cancer.

In our series positive results for HPV were strongly associated with the presence of concurrent CIN. In resource poor countries like India, frequent rounds of screening by cytology are not possible and therefore screening by HPV DNA testing may be a reasonable alternative to cytology. “Once in life time” screening at 30 – 35 years of age for HPV DNA combined with a direct clinical intervention shall result in a substantial reduction in cervical cancer. In future

perhaps cervical cancer screening with shift from a morphology based approach to one in which search for HPV becomes the focus of disease detection.

CORRESPONDENCE TO

Prof. Anuradha Khanna N8/180-AK 1, Rajendra Vihar
Nevada Varanasi – 221 005, INDIA Fax : 91-542 – 2367568
Email : dr_anuradhakhanna@yahoo.co.in

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Author Information

Anuradha Khanna, MD

Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University

Arti Matah, MD

Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University

Mohan Kumar, MD

Department of Pathology, Institute of Medical Sciences, Banaras Hindu University