Research Article

Human papiloma virus testing in the cervix of high-risk women: A hospital-based clinicopathological, colposcopic, and cytogenetic study

Subhash Bhardwaj[1], Farooq Ahmed Wani[2], Altaf Bandy[3]

[1]Department of Pathology, Govt. Medical College, Jammu, Jammu and Kashmir, India.
[2]Department of Pathology, College of Medicine, Aljouf University, Sakaka, Saudi Arabia.
[3]Department of Community Medicine, College of Medicine, Aljouf University, Sakaka, Saudi Arabia.
Correspondence to: Farooq Ahmed Wani, E-mail: wani_farooq786@yahoo.com

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Abstract

Background: The role of human papillomavirus (HPV) in the etiopathogenesis of precancerous and cancerous lesions of the cervix has been proven beyond doubt. Uterine cervix is a privileged organ being accessible and easily examined for lesions that can be extirpated in noninvasive stage, thus affecting a complete cure. Many different modalities for early detection of cervical lesions have been adopted, the most cost-effective being the Pap smear (Papanicolaou test). HPV DNA hybrid capture assays can be specifically used for detection of HPV DNA.

Objectives: The purpose of this study was to estimate HPV positivity in high-risk women and to evaluate its relationship with age, parity, and other risk factors. Besides, it was carried out to evaluate the results of cytology, colposcopy, histopathology and HPV hybridization in early detection of cervical carcinoma, and to evaluate each parameter in the present setup.

Materials and Methods: This was a hospital-based prospective study carried out from April 2006 to March 2007. Two hundred prospective patients were enrolled to study HPV positivity among high-risk women. Pap smears were taken and microscopically studied according to the Bethesda System 1988. Patients with atypical squamous cell of undeterminate significance (ASCUS) were then subjected to hybrid capture HPV test. Colposcopy examination and colposcopically-directed biopsies were taken in all the cases.

Results: Most of the patients with lesions suggestive of HPV belonged to younger age group (21–30 years), and there was a significant increase in epithelial abnormalities with advancing age ($p < 0.05$). Inflammatory smears were seen in 70 patients (35%); 40 patients (20%) reported as ASCUS were subjected to High Risk Capture II assay (for HPV 16, 18, 45, 56, 58) and high-risk HPV DNA was found in 20 (50%) of the patients. The sensitivity of cytology was only 61%, histopathology could detect 24 (12%) additional cases of dysplasia compared to cytology, indicating a low sensitivity of Pap smear. Colposcopy correlated well with histopathology with a comparatively high sensitivity (80%).

Conclusion: We conclude that cytology will continue to be a major screening method for detection of cervical lesions due to its low cost and easy availability. We also conclude that HPV DNA testing is a very sensitive and highly reproducible test but cannot be used as a mass screening procedure due to its expensive nature and its inaccessibility to the common masses.

KEY WORDS: Human Papillomavirus; Cervical Cancer; Pap Smear, Colposcopy

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Introduction

Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in both men and women worldwide.[1] More than 200 types of HPV have been recognized on the basis of DNA sequence data showing genomic differences.[2] Persistent infection with high-risk oncogenic HPV types is a major cause of cervical cancer.[3]

HPV DNA can be identified in nearly all specimens of invasive cervical cancer and in the majority (>95%) of the immediate cervical cancer precursors, namely high-grade squamous intraepithelial lesions (HSILs)—also known as cervical intraepithelial neoplasia 3 (CIN III) or carcinoma *in situ*.[4,5]

Worldwide, the most prevalent high-risk strains are HPV 16 and 18.[6] High-risk types HPV-16 and HPV-18 are responsible for 70% of cases of cervical cancer.[7] Low-risk HPVs, principally -6/11, are predominantly involved in the development of genital warts.[8]

In general, HPV infections tend to be transient and of relatively short duration in both young and old women, and within 8–24 months, 91% of HPV-infected women become HPV negative. The duration of infection is longer and sustained in women infected with high risk types of HPV and with sexual promiscuity.[9] HPV lesions have been subjects of intense study because of their appearance in very young women and their association with dysplasia and carcinoma of cervix.[10]

Since the introduction of Papanicolaou staining in 1949, cervical cancer incidence and mortality rates have declined steadily.[11] With time, screening for carcinoma of cervix has witnessed many modalities including colposcopy and cervical biopsy. HPV DNA detection by various methods such as fluorescent *in situ* hybridization, PCR, and hybrid capture assays has further revolutionized the detection of HPV infections quite early before the lesions are visible clinically.[12]

This study is an endeavor to evaluate all the parameters for early diagnosis of carcinoma of cervix, keeping in view the availability, acceptance, cost effectiveness, specificity, sensitivity, and other factors in our setup and therefore reach a consensus as to which method can be used as a mass screening procedure in our attempts to detect carcinoma of cervix at the earliest.

Material and Methods

This was a hospital-based prospective study carried out in collaboration with Department of Obstetrics and Gynecology, SMGS Hospital and Department of Pathology, Govt. Medical College, Jammu. Data collection started in April 2006 and continued for 12 months.

Sample

Two hundred prospective patients attending Department of Obstetrics and Gynecology, SMGS Hospital, were enrolled to study HPV positivity among high-risk women. Gynecology department assessed the patients and collected the Pap smear (Papanicolaou test) and did colposcopic examination. Pathology department examined the Pap smears and did histopathological examination of the biopsies taken.

Selection criteria: Sexually active women aged between 18 and 60 years fulfilling any of the following criteria:

Inclusion criteria:

a) Younger age of consummation
b) Multi-parity
c) Multiple sexual partners
d) Blood-stained discharge, contact bleeding, or intermenstrual bleeding
e) Vaginal discharge of any type not responding to drugs
f) Dyspareunia
g) Pain in lower abdomen and back
h) Pruritis

Exclusion criteria:

a) Pregnant women
b) Patients having frank malignancy
c) Patients having active genital infection

Patients fulfilling the above criteria were followed by a thorough general physical examination and local examination. Visual inspection of cervix was carried out to see any gross lesion or a visible growth. Cervical smears were taken with the help of Ayres wooden spatula in suspicious cases. The cytological smear was microscopically examined and reported according to the Bethesda System 1988.[13] Patients who reported back with an abnormal pap as suspicious, that is, atypical squamous cell of undeterminate significance (ASCUS) were then subjected to hybrid capture HPV test. Colposcopically-directed biopsies were taken and colposcopic index given by Reid and Stanhope[14] was used. Technique for taking a cytological smear is described in Annexure 1, colposcopy procedure is explained in Annexure 2, and HPV DNA capture is explained in Annexure 3.

Data analysis: Data were entered in SPSS, version 17. Appropriate statistical tests such as percentages and χ^2-tests were used to detect any significant association.

Results

This was a prospective study of cervical Pap smear examination of high-risk patients that attended the gynecology department of SMGS Hospital, Jammu. Women who were sexually active and fulfilled any of the inclusion criteria were recruited in the study. A total of 200 high-risk patients formed our study sample. There were 32% of patients <30 years and an equal number of patients contributed to 31–40 years age group; 35% patients were in the age group above 40 years.

There was a significant increase in HPV infection without intraepithelial abnormalities with advancing age ($p < 0.05$). The rate of intraepithelial lesions suggestive of HPV infection was highest in <30 years age group and decreased as the age progressed whereas that in Negative for intraepithelial lesions suggestive of HPV infection increased from 27% in <30 years to 56% in >40 years age group (Table 1).

Similarly, a significant increase was seen in HPV infection with increasing parity ($p < 0.05$). The rate of Negative for intraepithelial lesions suggestive of HPV infection increased from 36% for parity three or less to 66% among women of parity four or more (Table 2).

Table 1: Age groups and cytology

Age (years)	With intraepithelial lesions suggestive of HPV infection Number (%)	Negative for intraepithelial lesions suggestive of HPV infection Number (%)	Total Number (%)
<30	38 (48.7)	27 (22.13)	65 (32.5)
31–40	26 (33.3)	39 (32)	65 (32.5)
>40	14 (17.9)	56 (46)	70 (35)
Total	78	122	200

χ^2: 20.998, $p < 0.05$

Table 2: Parity and cytology

Parity	With intraepithelial lesions suggestive of HPV infection Number (%)	Negative for intraepithelial lesions suggestive of HPV infection Number (%)	Total Number (%)
<3	40 (51.3)	41 (33.6)	81 (40.0)
>4	38 (48.7)	81 (66.4)	119 (60.0)
Total	78	122	200

χ^2: 6.16, $p < 0.05$

Table 3: Age at consummation and cytology

Age (years)	With intraepithelial lesions suggestive of HPV infection Number (%)	Negative for intraepithelial lesions suggestive of HPV infection Number (%)	Total Number (%)
<20	38 (48.7)	46 (38)	84 (68.8)
20–25	26 (33.3)	36 (29.5)	62 (31)
>25	14 (28)	40 (32.7)	54 (44.2)
Total	78	122	200

χ^2: 5.47, $p > .05$

The highest prevalence of 48.7% of HPV infection was seen among women where the age of consummation was less than 20 years, explaining the prolonged exposure as main important reason for intraepithelial lesions suggestive of HPV infection. But the results were statistically insignificant ($p > 0.05$) (Table 3).

Women who were illiterate and belong to low economic group showed a higher prevalence of HPV positivity as compared to those who were literate and belonged to high economic group.

Pap smears were evaluated using Bethesda System 1988. Forty cases (20%) appeared to be normal and ten cases (5%) could not be reported due to the field obscured by blood. Inflammatory smears were seen in 70 patients (35%). ASCUS was observed in 40 (20%). Low-grade squamous intraepithelial lesions (LSIL) and HSIL comprised 34 (17%) and 6 (3%) cases, respectively (Figure 1).

Forty patients (20%) reported as ASCUS were subjected to High Risk Capture II assay (for HPV 16, 18, 45, 56, 58) and high risk HPV DNA was found in 20 (50%) patients.

All the 200 patients enrolled for the study were subjected to colposcopic examination and colposcopically directed biopsies were taken. On colposcopy, 42 patients showed no abnormality. Approximately 120 patients were diagnosed as having subclinical papillomavirus infection with Reid's colposcopic score (RCS) of 0–2. RCS of 3–5 was obtained in 16 patients whereas RCS of 6–8 was obtained in 11 patients (Figure 2). Colposcopy detected 10 lesions of trichomoniasis and 1 case of herpes as compared to cytology that detected 4 cases of trichomoniasis only.

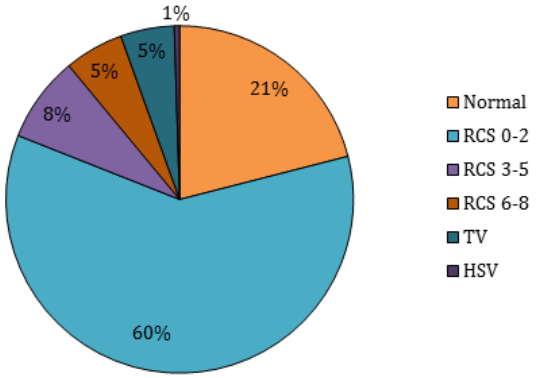

Figure 1: Colposcopic findings in the study group.

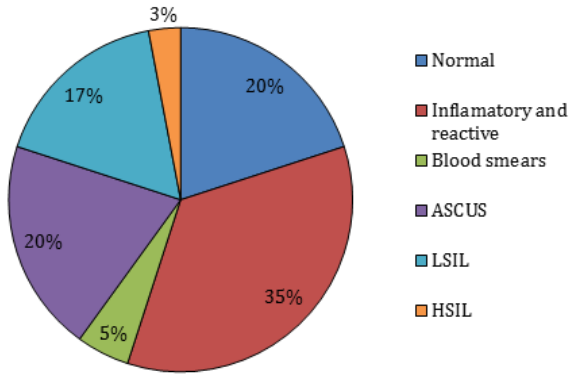

Figure 2: Predominant features in Pap smear.

Table 4: Correlation of cytology and histopathology

Cytology	Histopathology		Total
	Positive	Negative	
Positive	38	42	80
Negative	24	96	120
Total	62	138	200

Sensitivity: 61.3%, positive predictive value (PV+): 47%, specificity: 69.6%, negative predictive value (PV–): 80%

Table 5: Correlation of colposcopy and histopathology

Colposcopy	Histopathology		Total
	Positive	Negative	
Positive	50	14	64
Negative	12	124	136
Total	62	138	200

Sensitivity: 80.6%, positive predictive value (PV+): 78%, specificity: 89.9%, negative predictive value (PV–): 91%

The sensitivity of cytology was only 61%; histopathology could detect 24 (12%) additional cases of dysplasia compared to cytology, indicating a low sensitivity of Pap smear. Colposcopy correlated well with histopathology with a comparatively high sensitivity (80%). It was only in 6% of cases that colposcopy could not detect the underlying lesion (Tables 4 and 5).

Discussion

The recognition that cervical cancer is a result of an unresolved genital infection by some types of HPV was a major discovery in human cancer etiology.[5] By the year 2000, epidemiological evidences had amassed a large and consistent body of evidence that showed a solid and specific association between HPV infections and cervical cancer beyond reasonable doubt. This was also proved that precursor lesions are identifiable years before the appearance of invasive carcinoma, and so the importance of screening programs can be realized.

The HPV is responsible for 95%–100% of the cervical carcinoma cases, which is the second most prevalent malignant neoplasm among women worldwide.[3,15,16]

A total of 200 high-risk patients formed our study sample. Incidence of intraepithelial lesions suggestive of HPV infection was highest in <30 years age group. This was followed by a progressive decrease of incidence as age advances. Clavel et al.[17] found the peak incidence of infection in third decade of life (23.6%) with a progressive decrease after 30 years. Sardana et al.[18] also found maximum incidence of infection in the 21–30 years age group with decreased incidence as age advanced. The incidence of Negative for intraepithelial lesions suggestive of HPV infection increased from 27% in <30 years to 56% in >40 years age group with a significant *p*-value of <0.05.

We also found that prevalence of Negative for intraepithelial lesions increased as parity increased. Sardana et al.[18] also observed increased frequency with increasing parity and found the frequency of infection of different parity groups statistically significant. We found the highest prevalence of HPV infection (48.7%) among women where the age of consummation was less than 20 years, explaining the prolonged exposure and immature cervix as main important factor for HPV infection. Swan and Brown[19] showed that 14.5% of their patients were less than 17 years of age when exposed to sex and found age at regular intercourse to be an important risk factor.

There was a positive correlation between HPV infection and illiteracy and low income status. Similar results were reported by Sardana et al.[18] in their epidemiological survey.

Cytological reporting was carried out according to the Bethesda System 1988. Inflammatory smears were seen in 70 patients (35%). LSIL and HSIL comprised 34 (17%) and 6 (3%) cases, respectively. ASCUS was found in 40 cases (20%) of the patients. (Figure 3). Kurman et al.[20] state that ASCUS should not exceed 10% of the smears but believed that false-positive rate can be 20%–30%. Dr. Alex Ferenczy of Mc Gill University, Canada, found false-positive rates to be 5%–70%.[12] We attribute the slight increase in our study due to drying artifacts and indistinguishable inflammation-induced atypia.

Forty patients (20%) reported as ASCUS were subjected to High Risk Capture II assay (for HPV 16, 18, 45, 56, 58), and high-risk HPV DNA was found in 20 (50%) patients. Clavel et al.[21] found 55.9% of ASCUS patients to be HR-HPV positive. Sherman et al.[22] also found high-risk HPV positivity in 50% of patients with ASCUS.

On colposcopy, subclinical papillomavirus infection was seen in 60% of the patients. Only 8% patients had an RCS of 3–5 and 5.5% had a score of 6–8 whereas 21% of patients had normal colposcopy. CIN I was seen in 120 patients (60%) with colposcopy. Out of HSIL lesions encompassing CIN II/III (Figure 4), cytology could detect only 3% of lesions as compared to 5.5% of lesions detected by colposcopy.

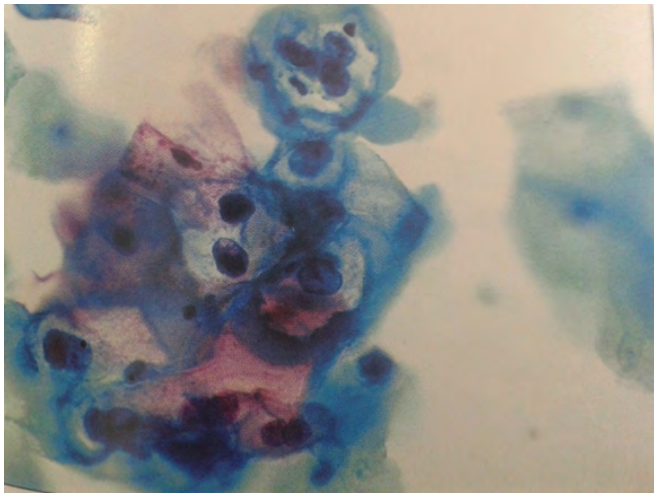

Figure 3: Photomicrograph showing atypical squamous cells in Pap smear (Pap, 600×).

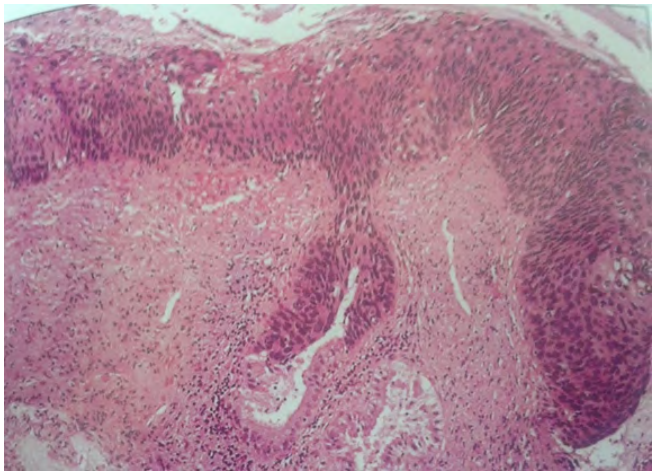

Figure 4: Photomicrograph showing cervical intraepithelial neoplasia II (CIN-II) on histopathology (H&E, 400×).

Histopathology could detect 24 (12%) additional cases of dysplasia that were cytologically normal, thus Pap smear was seen to have sensitivity of 61.3% as compared to histopathology in detecting HPV infection. The low sensitivity of Pap smear has also been revealed by Khanna et al. who found a sensitivity of 58% as far as cytology was concerned.[23] Out of HSIL lesions encompassing CIN II/III (Fig-4). Clavel C. et al had found the sensitivity of conventional Pap smear to be approximately 57.7%. He however found an improved sensitivity of about 73.2% with thin liquid preparation cytology.[17] Many other studies have found the sensitivity of Pap smears to be low often below 50%.[24,25]

Colposcopy correlated well with histopathology. The sensitivity of colposcopy was found to be 80.6% with a specificity of 89.9%. It was only in 6% of cases that it could not detect the underlying lesion. Gehlot found that the colposcopic prediction of histopathology was clinically accurate in 85% of their cases.[26] Seshadri et al 1990 show a similar correlation of 87.6% between colposcopy and histopathology.[27] Khanna et al.[23] had shown cytology to be 58% sensitive and colposcopy to be 92.8% sensitive in detecting HPV lesions. In a meta-analysis by Mitchell et al.,[28] the sensitivity of conventional colposcopy was found to be high in the range of 64%–99%.

Thus, colposcopy has emerged as a better screening option than conventional Pap smear. The two limitations of it are its cost and expertise needed to interpret it, but once installed after one-time investment, it can be used to detect lesions and perform biopsies of suspicious lesions at the earliest.

HPV DNA testing is being increasingly adopted as a clinical management tool in women with ASCUS cytology. The ASCCP Consensus Conference 2001 in Bethesda, USA, recommended that "A program of repeat cervical cytological testing, colposcopy or DNA testing for HR HPV types are all acceptable methods for managing women with ASCUS. When liquid-based cytology is used or when co-collection of HPV DNA testing can be done, reflex HPV DNA testing is the preferred approach".[29] Lörincz and Richart[30] in a systematic review concluded that HPV DNA testing was a more sensitive indicator for prevalent high-grade CIN than either conventional or liquid cytology.

In a developing country like ours, routine HPV DNA testing is neither cost-effective nor acceptable. One of the principle barriers is the cost of HPV test as compared to the cytology and histopathology. Routine HPV testing is more expensive even in technically advanced societies. It seems extremely unlikely that health-care systems in developing countries can deliver effective HPV testing where effective delivery even of Pap screening has not been accomplished yet.

Conclusion

We conclude that cytology will continue to be a major screening method for detection of cervical lesions but it needs a good technical approach and a high professional skill to be more sensitive.

Although colposcopy emerged as a better screening tool than the conventional Pap smear, but due to its cost and expertise required to interpret it, it cannot be used as a mass-screening tool.

We also conclude that HPV DNA testing is a very sensitive, highly reproducible test but in a developing country like ours, it cannot be used as a mass screening procedure due to its expenses, unavailability, and its inaccessibility to the common masses.

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