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Human papillomavirus (HPV) infection and abnormal cervical cytopathology among human immuno-deficiency virus (HIV) positive women in Northern India

Pooja Pundhir[1]*, Y. M. Mala[1], R. Tripathi[1], B. C. Das[2] and S. Bhambani[2]

[1]Department of Obstetrics and Gynaecology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi, India.
[2]Institute of Cytology and Preventive Oncology, Indian Council of Medical Research (ICMR), Delhi, India.

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Human immune deficiency virus (HIV) infection is associated with a higher risk of human papillomavirus (HPV) positivity and cervical intraepithelial neoplasia (CIN) which may progress to cancer. Hence, it is important to screen all HIV positive women for this cancer which is amenable to cure in early stages. A cross sectional case-control study was conducted on 100 HIV seropositive women and matched seronegative controls attending Lok Nayak Hospital, New Delhi to study the prevalence of HPV infection and abnormal cervical cytopathology. A detailed cervical evaluation including a per-speculum examination, pap smear, cervical scrape for HPV DNA polymerase chain reaction (PCR), visual inspection of cervix with acetic acid, colposcopy and guided biopsy were done. Abnormal pap smears were found in 63% women of which 10% were squamous intraepithelial lesions. Biopsy revealed cervical intraepithelial neoplasia in 2% of all cases (5.6% of all patients biopsied). Prevalence of HPV in HIV positives was 24% compared to 4% in controls (p value 0.001), also bearing a significant correlation with CIN, thus placing HIV positives at a higher risk for cervical dysplasias and cancer. The sensitivity of HPV DNA test for detection of cervical dysplasias was 86.3% and specificity 64.2%. HIV positivity predisposes to invasive cervical cancer on account of immunosuppression and co-existing HPV infection, thereby the need for aggressive screening for cervical intraepithelial lesions. Both HPV DNA PCR and pap smear are optimal screening tools in these women.

Key words: Human immune deficiency virus (HIV), cervical human papillomavirus (HPV) infection, cervical intraepithelial neoplasia, pap smear, human papillomavirus (HPV) DNA polymerase chain reaction (PCR).

INTRODUCTION

India is one of the most populous countries in the world with more than one billion inhabitants. Of this number, it is estimated that about 2.4 million Indians are living with human immune deficiency virus (HIV), the national audit prevalence being 0.36%. The proportion contributed by women is 39%. Both HIV serotypes exist in India and HIV1-C is the most predominant. In a setting of expanding feminization of the HIV epidemic, Indian women are overburdened with a high morbidity and mortality and limited access to health care. HIV positivity and cervical neoplasia are both prevalent health problems in India, the latter being the most common genital tract cancers in women in India, with a significant prevalence of precursor intraepithelial lesions in the reproductive age group. This

*Corresponding author. E-mail: poojapundhir10@gmail.com. Tel: 469-279-3622.

is thus one of the few cancers which can be subject to primary prevention by screening tests. Cervical screening tests should be available to the general population, and especially to HIV positive women, who are at an increased risk of developing cervical cancer. It is well accepted that women with AIDS show a high prevalence for cervical cancer. Cervical cancer has been since long accepted as an AIDS defining diagnosis by CDC on account of the high prevalence (Centers for Disease Control and Prevention, 1993).

HIV infection is associated with a higher risk of human papillomavirus (HPV) positivity and consequent cervical intraepithelial lesions (CIN) which may progress to cervical cancer. Papillomaviruses are small non-enveloped double stranded DNA viruses. Their genome includes two important genes, E6 and E7, which produce proteins that can attach themselves to Rb and p53 tumor suppressor genes and abrogate their functions. The high risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68) which are responsible for cervical neoplasias have cytologic effects ranging from mild form of the disease, condylomatous atypia or koilocytic atypia to severe dysplasias. HPV 16 has been found to be the most common type and appears to begin progression earlier and more consistently than the other subtypes. This is further compounded by the immunosuppression in HIV positive women resulting in chronic persistent HPV infection especially with several strains simultaneously, which is more likely to progress to invasive cancer.

In developed countries, on account of this high rate of dysplasia, a comprehensive gynecologic examination including a pap smear has been indicated at the initial evaluation and at subsequent visits. In view of the dual burden of HIV infection and cervical cancers in India, locally feasible screening guidelines need to be developed based on accurate data on prevalence of cervical abnormalities and HPV infection among HIV positive women. This will help to diagnose the maximum possible HIV positive women in early stages of CIN and reducing morbidity on account of CIN and cancer in this group of patients.

MATERIALS AND METHODS

Recruitment of the study population

This was a cross-sectional case control study conducted in the Department of Obstetrics and Gynecology, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi from September, 2007 to February, 2009. One hundred HIV seropositive women attending the antiretroviral therapy (ART) Clinic at Lok Nayak Hospital and an equal number of healthy HIV seronegative women, matched for age and parity, taken as controls, were recruited. Patients with a surgically or congenitally absent cervix, known cervical malignancy, or an associated pregnancy were excluded from the study. A written informed consent to participate in the study was solicited following which the cases were subjected to a complete general physical examination, a local genital examination and a per speculum examination.

Screening and sample collection

A pap smear was taken for cytological examination using the wooden Ayre's spatula, was stained by the Papanicolaou's method and graded according to the modified Bethesda system. The same end of the Ayre's spatula was preserved in phosphate buffer saline (PBS 7.2) at -40°C for DNA extraction and HPV genotyping for types 6, 11, 16, 18 (the most common types genotypes) by polymerase chain reaction (PCR) using MY09/MY11 consensus primer (Milutin Gasperov et al., 2008). The cervix was visualized after liberal application of 5% acetic acid (VIA). Any whitening of the original pinkish color of a normal cervix was considered VIA positive whereas absence of the same was considered VIA negative. All subjects were then subjected to a colposcopic examination, the findings of which were noted according to the International Colposcopic nomenclature and scoring was done using modified Reid's index. A cervical biopsy was taken from suspicious areas, fixed in 10% formalin and sent for histopathological examination.

Statistical analysis

All data was compiled in terms of counts (percentage) under different categorical variables. The correlation between two categorical variables was determined using Chi square test or Fischer's exact test. The sensitivity, specificity, positive predictive value, negative predictive value were calculated for HPV DNA taking cervical biopsy as the standard reference.

RESULTS

Socio-demographic characteristics of the study population

The women in the study belonged to the reproductive age group. The mean age was 30.98 ± 5.8 standard deviation (SD) years for cases and 30.69 ± 3.1 SD years for controls. The mean parity of cases was found to be 2.41 ± 0.9, compared to 2.79 ± 0.8 in the control group. The most common mode of transmission of HIV infection amongst the study group was the heterosexual route (90%). None of the HIV seropositive women gave history of multiple sexual partners, though a history of promiscuity was elicited from their husbands. Most of the women studied (41%) were in World Health Organization (WHO) stage I and the mean CD4 cell count was 383.89/µl. Fifty five percent were already on anti-retroviral treatment at the time of the study.

Cervical cancer screening

On per-speculum examination, the cervix was found to be abnormal in 43% of the HIV positive women as compared to 8% of controls ($p < 0.001$). The most common finding was cervical erosion seen in 20% of cases followed by vaginal discharge in 13%. HIV positive women were found to have a significantly greater percentage of abnormal pap smears ($p < 0.01$) (Table 1). A statistical significance was found between abnormal pap smear findings and advancing WHO stage ($p < 0.015$) and lower

Table 1. Results of cytological examination (Pap smear).

Pap test report	Cases (%)	Controls (%)
Normal	37	89
Inflammation	28	10
Inflammation with TV/ *Candida*/ HSV/ *B. vaginosis*	11	0
Inflammation with HPV	14	1
ASCUS	2	0
LSIL	0	0
LSIL with HPV	4	0
HSIL	2	0
Inadequate	2	0

TV- Trichomonas vaginalis, HSV- herpes simples virus, ASCUS- atypical cells of undetermined significance, LSIL- low grade squamous intraepithelial neoplasia, HSIL- high grade squamous intraepithelial neoplasia, HPV- human papillomavirus.

CD4 cell counts ($p < 0.016$). On application of 5% acetic acid to the cervix, 33% were VIA positive compared to only 3% controls ($p < 0.001$). Colposcopy was found to be abnormal in 36% of HIV seropositive patients and 1% of seronegative controls ($p < 0.001$) and majority (52.7%) had a Reid score of 3 to 5. A colposcopy guided biopsy was indicated in 36 cases whereas none of the controls were eligible for a cervical biopsy by virtue of normal colposcopic examination. The most common finding was the presence of condylomatous changes (suggestive of HPV) infection in 55.6% of cases. High grade lesions (CINIII) were seen in 5.6% of the patients biopsied, that is, 2% of the total cases.

Cervical HPV positivity in HIV positive women

HPV positivity on cervical smear was seen in 24% of HIV seropositive women compared to 4% of controls (Tables 2 and 3). Most of the HIV positive subjects who tested positive for HPV were almost equally distributed among WHO stages I, II, and III. There was no significant correlation between the WHO stage of HIV and HPV infection ($p = 0.184$). A statistical significance was found between HPV positivity and low CD4 counts ($p = 0.001$). Eighty seven percent of HPV positive cases were VIA positive compared to 15.8% of HPV negative cases (p 0.001). Most (66.7%) of the HPV positive cases had a Reid score between 3 to 5 while 80% of HPV negative cases revealed a normal colposcopic examination ($p < 0.001$) (Table 4). The sensitivity of HPV DNA test for detection of cervical dysplasias in HIV positive women was 86.3% and specificity 64.2%. The positive predictive value was 79.1% and negative predictive value 75%.

DISCUSSION

Acquired immune deficiency syndrome (AIDS) in our country is most prevalent among sexually active younger population groups. The majority of HIV infection (87.7%) afflicts women between 15 to 44 years of age. The cervix was found to be grossly unhealthy on per-speculum examination in 43% cases and only 8% controls. Amongst HIV positive women, cervical erosion was the most prevalent finding (20%), followed by the presence of vaginal discharge (13%), and a suspicious looking cervix (10%). Thus, in the present study, HIV seropositive women showed a higher incidence of sexually transmitted infections (STIs) manifesting as cervical erosion and vaginal discharge. Out of them, 45% showed inflammatory pap smears and 10% showed squamous intraepithelial neoplasia (SIL) and only 20% were normal smears. Infection and erosion heal by a process of squamous metaplasia, and it is this area, that is the transformation zone which is most susceptible to dysplastic changes, thus making these patients predisposed to development of CIN. Sixty six percent of HIV positive patients with an unhealthy cervix had SIL on cytology and CIN on histopathology. Hence, an abnormal cervix on per-speculum examination is likely to harbor an underlying cervical intraepithelial abnormality.

In our study, 63% of cases had an abnormal pap smear as compared to 11% of controls. Fourteen percent of smears showed koilocytic changes suggestive of HPV infection. Two percent of pap smears had atypical cells of undetermined significance (ASCUS). The percentage of cases with SIL was 6%, 4% being low grade squamous intraepithelial neoplasia (LSIL) with HPV, and 2% high grade squamous intraepithelial neoplasia (HSIL).

In the present study, the percentage of HIV positive women with CIN on histopathology was 2%. The histopathological examination of both patients revealed CIN III with condylomatous changes. Of the remaining cases, 20% were found to have condylomatous changes. A biopsy was not indicated in any of the controls and thus a statistical significance could not be determined. Thus, the percentage of CIN in HIV positive women was found to be higher, comparing with data in studies conducted in the general Indian population where the prevalence of

Table 2. Results of HPV DNA PCR test.

Parameter	Negative (%)	Positive (%) (HPV type)				
		6	11	16	18	Total HPV positives
Cases (n=100)	76	1	0	22	1	24
Controls (n=100)	96	0	0	4	0	4

Presence of HPV DNA in HIV positive women was found to be statistically significant (p = 0.001). Most common type was HPV 16

Table 3. Association of HPV infection with Pap smear results in HIV positive women.

Parameter	Normal [N (%)]	Inflammation [N (%)]	Inflammation with HPV [N (%)]	ASCUS [N (%)]	LSIL [N (%)]	LSIL with HPV [[N (%)]	HSIL [N (%)]
HPV positive (n=24)	2 (8.3)	5 (20.5)	10 (41.7)	1 (4.2)	0 (0)	4 (16.7)	2 (8.3)
HPV negative (n=76)	36 (47.4)	34 (46.1)	4 (5.3)	1 (1.3)	0	0	0

Of the 24 HPV positive cases, 25% showed dysplastic cells and 41.7% showed inflammation with HPV on Pap smear. The association of HPV positivity with abnormal Pap smear was found to be significant (p < 0.001)

Table 4. Association of CIN with HPV positivity in HIV positive cases.

Parameter	HPV positive (%)	HPV negative (%)
Chr. Cervicitis (n=14)	4 (28.5)	10 (71.5)
Condylomatous changes (n=20)	17 (85)	3 (15)
CIN I (n=0)	0	0
CIN II (n=0)	0	0
CIN III (n=2)	2 (10)	0

Total number of cervical biopsies taken = 36. 85% patients with condylomatous changes were positive for HPV DNA whereas all patients with CIN III were harbouring HPV.

CIN was found to range from 0.47 to 1.3% (Mulay et al., 2009). No cases with invasive cervical cancer were detected in our study. This could be explained by the fact that cervical cancer is a disease mainly of elderly groups and the mean age of cases in our study was 30.98 years.

Studies in various regions of the world have shown that women infected with HIV are at an increased risk for cervical cancer precursor lesions. The percentage of HIV positive patients with CIN is variable but has been reported to be as high as 30% in African studies where the prevalence of HIV itself is quite high (Christopher et al., 2007). Weissborn et al. (2003) found HIV infection to be associated with significantly high HPV loads and cervical dysplasia, with the highest load in advanced disease. Massad et al. (2005) conducted a large prospective multicenter cohort study and found at least one abnormal smear during follow-up of 73% of HIV positive women and 42% seronegatives (Luthra et al., 1987). The lower percentage in our study could be attributed to small sample size and the lower prevalence of HIV amongst the population covered in the study. This may also be explained by most patients belonging to WHO stage I of HIV disease and others were already on antiretroviral therapy (ART). The development of CIN in HIV positive patients may also be influenced by other factors like parity, nutritional status, hygiene, ART, immediate treatment after diagnosis of HIV, and presence of other STIs. The patients with CIN III in our study were planned for a cone biopsy and further follow-up. A significant correlation was found between the prevalence of CIN and clinical WHO stage or the degree of immunosuppression (CD4 cell count). Advanced HIV related disease which is associated with immunosuppression favors persistence of high loads of cervical oncogenic type HPV infection and clinical expression in HIV positive women.

A greater prevalence of HPV infection has been found in HIV positive women. The prevalence ranges from 30% to as high as 65% (Maria et al., 2008; Danny et al., 2008). In our study, 24% of HIV infected women were positive for HPV as compared to 4% of controls. While we did not find any association of HPV infection with WHO stage of disease, there was a significant correlation with lower CD4 counts. In a study conducted by National AIDS Research Institute (NARI), Pune in 2003, pap smear abnormalities were found in 6.3% of the 287 HIV

Table 5. Comparison of sensitivity and specificity of high risk HPV for screening CIN.

Study	Number	Sensitivity (%)	Specificity (%)
Kaufman et al. (1997)	462	52	-
Infantlolino et al. (2000)	314	86	41.3
Cuzick et al. (1995)	2009	75	-
Gaffikin et al. (2003)	2199	80	61
Present study	100	86.3	64.2

positive women screened while 33% were positive for HPV 16/18 (Joshi et al., 2005). Greater HPV positivity in HIV positive women is attributed to various factors including chronic immunosuppression and consequent chronic HPV infection, infection with strains which are more likely to cause cancer, HPV in both the cervix and anus, infection with several strains of HPV virus simultaneously, reactivated HPV infections which were previously under control and HPV that poorly responds to other therapies-multiple treatments using different methods may be needed.

HIV infected women with co-existing HPV were analyzed and a significantly greater prevalence of abnormal cervical cytology was found. Of the 24 HPV positive cases in our study, 25% showed dysplastic cells on pap smear (LSIL with HPV, HSIL). Forty two percent revealed inflammation and HPV, and the remainder comprised of normal and simple inflammatory smears. Abnormal pap smears and colposcopy, and VIA positivity were common in HPV positive cervical smears. Screening for HPV infection and CIN in HIV positive women using VIA and colposcopy should be an early adjunct to pap smear considering the high risk of cervical dysplasias in HIV positive women.

The validity of an HPV test (to screen for cervical dysplasias in HIV positive women) calculated in our study was comparable with that in previous studies where the sensitivity ranges from 50 to 80% and specificity is 40 to 60% (Table 5). Combining the HPV DNA test with pap smear examination increases the negative predictive value to nearly 99%.

Conclusions

It is evident from the present study that HIV positive women are overwhelmingly burdened with a greater risk of cervical neoplasia. Higher rates of HPV infection with a greater persistence and severity of sequelae are evidence for an early diagnosis and interruption of the infectious pathology conducive to cervical cancer. Detection of HPV infection can be employed as a primary preventive measure for cervical cancer by direct PCR or indirect tools for example, pap smear, VIA, colposcopy, either singly or as combined integrated tests. The prevalence of CIN and high risk HPV infection in HIV seropositive women is high enough to warrant a routine gynecological evaluation and cervical cytological screening in these patients. However, larger and longitudinal studies are needed to evaluate the progression and effects of antiretroviral therapy on these abnormalities as well as to choose the best screening tool in HIV seropositive women.

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ABBREVIATIONS

ART, Anti-retroviral therapy; **ASCUS,** atypical squamous cells of undetermined significance; **AIDS,** acquired immunodeficiency syndrome; **CDC,** Center for Disease Control; **CIN,** cervical intraepithelial neoplaisa; **DNA,** deoxyribonucleic acid; **HIV,** human immunodeficiency virus; **HPV,** human papilloma virus; **SIL,** squamous intraepithelial neoplasia; **LSIL,** low grade squamous intraepithelial neoplasia; **HSIL,** high grade squamous intraepithelial neoplasia; **HSV,** herpes simplex virus; **NARI,** National AIDS Research Institute; **PCR,** polymerase chain reaction; **STI,** sexually transmitted infections; **TV,** trichomonas vaginalis; **VIA,** visualisation with acetic acid; **WHO,** World Health Organisation.

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