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Original Research Article

A study on HPV mRNA test and colposcopy in HIV positive women for early detection of cervical intraepithelial lesions

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ABSTRACT

Background: Sexually transmitted infections (STIs) have a profound impact on sexual and reproductive health worldwide. At any point of time, globally more than 290 million women suffer from HPV infection, one of the most common STIs. There are only few studies from India reported regarding the prevalence of HPV and cervical abnormalities among HIV-positive women. This study was carried out to estimate the prevalence of HPV and other genital tract infection in HIV positive women and correlate HPV mRNA test and colposcopic findings with cyto-histopathology in HIV positive women who are not on antiretroviral therapy (ART). Secondary objective was to benefit HIV positive women by making them aware about the importance of screening, early detection and treatment of various lower genital tract infections, CIN and cancer cervix.
Methods: cross sectional observational study was conducted in department of Obstetrics and Gynecology, NSCB medical college Jabalpur (M.P.) from March 2015 to August 2016 on 70 HIV positive women in whom anti-retroviral therapy was not started. This study was approved by institutional ethical committee. The data was recorded in predesign coded case report form and statistical analysis was performed using the STATA 12.1.
Results: In the present study, prevalence of lower genital tract infections in HIV positive women not on ART was 30.0% and the prevalence of high risk HPV was 5.9%.
Conclusions: The study concludes that diagnostic efficacy of HPV mRNA test has similar diagnostic value as that of histopathology report.

Keywords: CIN, Colposcopy, HIV, High risk HPV, HPV mRNA

INTRODUCTION

Sexually transmitted infections (STIs) have a profound impact on sexual and reproductive health worldwide. According to WHO factsheet 2016 more than 1 million STIs are acquired every day. Each year, there are estimated 357 million new infections with 1 of 4 STIs: chlamydia (131 million), gonorrhoea (78 million), syphilis (5.6 million) and trichomoniasis (143 million). More than 500 million people are living with genital HSV (Herpes Simplex Virus) infection. At any point in time, more than 290 million women suffer from HPV infection, one of the most common STIs.[1] STIs can have serious consequences beyond the immediate impact of the infection itself.

India has the third largest number of people living with HIV/AIDS (acquired immunodeficiency syndrome) and

accounts for nearly 1/4th of the global cervical cancer deaths. Of the 2.09 million people living with HIV/AIDS in India, 39 per cent are women.[2,3] There are only few studies from the country regarding the prevalence and types of HPV and cervical abnormalities among HIV-positive women. A hospital based study from the eastern part of India observed a prevalence of oncogenic HPV among HIV-positive women to be 46.2% whereas a study from northern India showed a prevalence of high-risk HPV in 20%.[4,5]

Justification

HIV positive women are at higher risk of developing reproductive tract infections as compared to HIV negative women. Large observational studies involving HIV positive women have demonstrated a strong and consistent association between confection with HIV, HPV and CIN. It is therefore important for the clinicians to screen women who are HIV positive for reproductive tract infections and CIN. Previously similar study in the department revealed high rate of regression of HPV infection in HIV positive women who were on ART.[6]

The present study is planned to know the prevalence of HPV and other lower genital tract infection in HIV positive women who are not on ART and also correlating colposcopic findings and HPV mRNA test results with cyto-histopathology reports in the study group.

METHODS

A cross sectional observational study was conducted in Department of Obstetrics and Gynaecology, NSCB medical college Jabalpur (MP), India from March 2015 to 31st August 2016 on 70 HIV positive women not yet started anti-retroviral therapy. Ethical approval was taken from institutional ethical committee.

All women above 18 years, who are sexually active and HIV positive not on ART irrespective of their pregnancy status were included in the study group. HIV positive women who were taking anti-retroviral therapy (ART), women with bleeding per vaginum and women with visible growth on Cervix were excluded from the study

HIV positive women were counselled for gynaecological examination and testing in the department of gynaecology. Total 96 HIV positive women were counselled and out of these 70 women gave consent for examination and testing. They were explained the procedure and informed consent was taken. The patient was taken on the colposcopy examination chair. Colposcopic examination (Karl Kaps Germany) of the perianal region and vulva was done. A self-retaining Cusco's speculum was applied. With the help of two cytobrushes, sample from ectocervix and endocervix was taken, Pap smear fixed with 95% ethanol and slides were sent to Pathology Department for examination and at the same time sample was placed in the HPV mRNA vial and was sent by courier to Cure Health Diagnostics Pvt Ltd. within 24 hours of collection (free samples were issued for research work). Cervix was cleaned with saline swab. Ectocervix and vagina was examined under magnification and green filter. Freshly prepared 5% glacial acetic acid solution was applied on the ectocervix (5ml of glacial acetic acid mixed with 95 ml of distilled water). As per IFCPC 2011 classification normal and abnormal findings were noted and scoring was done as per modified RCI index.[7,8] The cervix was divided into four quadrants by an imaginary line passing through the centre from 6'o clock to12'o clock position and from 3'o clock to 9'o clock position and Colposcopic examination of each quadrant was done in a clockwise direction starting from right upper quadrant and findings documented as equation by simplified RCI scoring method.[9] If the score was more than 2, then biopsy was taken and send for histopathology reporting in Department of Pathology, NSCB medical college, Jabalpur (MP). At later date pap test, HPV mRNA, Colposcopy findings and histopathology reports were noted and analysed.

Basic demographic characteristics clinical presentation and laboratory findings were recorded in predesigned case report from all studied cases. Numerically coded data entered in MS excel 2007 worksheet and logical validation and editing was done before analysis. Categorical variables were summarized as frequency distribution and mean (±SD) was analysed using chi square or fisher's exact test as appropriate. Sensitivity, specificity, positive and negative predictive values with 95% confidence interval was also calculated for diagnostics. Statistical analysis was performed using STATA 12.1.

RESULTS

The background characteristics of studied group revealed 51.4% women were between 31-39 years of age. 61.4% by occupation were belonging to rural area. 92.9% of women were Hindu by religion. 65.7% of women not educated.by occupation 65.7% of women were housewife. 37.1% women had marital life of less than 5 years and 34.3% had marital between 5-10 years. 30% women were para 2 and 28.6% were para 1 (Table 1). Majority 58.6% cases, presented with complaint of white discharge followed by 14.3% with itching, 11.4% with burning micturition, 5.3% with abdominal pain, 8.6% with irregular bleeding per vaginum, 4.3% with postcoital bleeding and 2.9% with other complaints. A case who was diagnosed as CIN 2 presented with complaint of itching over private parts and a case diagnosed as CIN 3 presented with complaint of irregular bleeding per vaginum (Figure 1).

Prevalence of lower genital infections in HIV positive women in the present study was 30% (n=21). Out of which 14.2% cases had candidiasis, 14.2% cases had trichomoniasis, 5.9% with high risk HPV infection, 4.7%

cases with vulval condyloma and 61% with cervicitis due to other causes (Figure 2).

Table 1: Demographic characteristics of the study group.

Variable	Frequency	%
Age (Years)		
20 – 30	25	35.7
31 – 39	36	51.4
40 – 49	06	8.6
> 60	03	4.3
Locality		
Rural	43	61.4
Urban	27	38.6
Religion		
Hindu	65	92.9
Muslim	05	7.1
Education		
Illiterate	46	65.7
Primary	11	15.7
Middle	08	11.4
Secondary	04	5.7
Graduate	02	1.4
Occupation		
Daily wage worker	20	29.6
Farmer	08	2.9
Service	08	2.9
Housewife	46	65.7
Duration of marriage		
< 5 years	26	37.1
5 – 10	24	34.3
11 – 20	12	17.1
21 – 30	05	7.1
> 31	03	4.3
Parity		
1	20	28.6
2	21	30
3	17	24.3
> 4	08	11.4

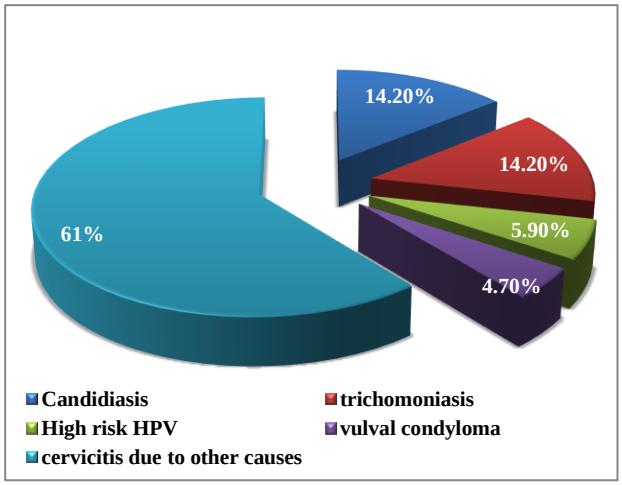

Figure 1: Prevalence of lower genital tract infections.

Majority 41.4% cases had CD4 count >500 cells/cumm, 52.9% cases had 200-499 cells/cumm and remaining 5.7% cases had <200 cells/cumm. There were 2 case with major colposcopic findings (CIN 2, 3) who had CD4 count was <200 cells/cumm.

On cytology 91.4% (n=64) cases revealed negative for intraepithelial lesion (NILM), 7.1% (n=5) cases revealed ASCUS and 1.5% (n=1) revealed LSIL. Out of 5 ASCUS one case revealed major colposcopic findings and 4 cases revealed minor colposcopic findings. One case reported LSIL revealed major colposcopic findings. This was found statistically significant (p=0.002).

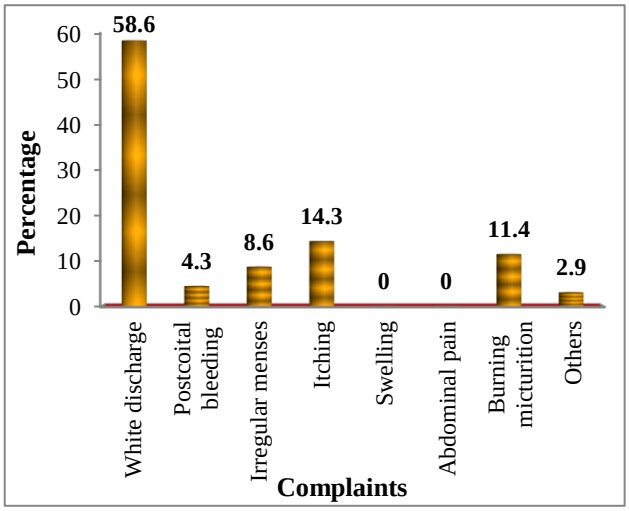

Figure 2: Distribution of cases according to presenting complaints.

In two cases with positive HPV mRNA testcolposcopy also detects the major colposcopic findings and was statistically significant. (p=0.002) and the same two cases histopathology confirmed the CIN2 and CIN3.This finding was statistically significant. (p value=0.002)

Sensitivity of colposcopy against HPV mRNA test is 100% (95% confidence interval 15.8%-100%)

Specificity of colposcopy against HPV mRNA test is 59.4% (95% confidence interval 40.6-76.3%). Positive predictive value of colposcopy against HPV mRNA test is 13.3% (95% confidence interval 1.7-4.5%)

Negative predictive value of colposcopy against HPV mRNA test is 100% (95% confidence interval 82.8-99.9%). Specificity and PPV of colposcopy was less in our study due to the reason that it detects the minor findings also. Sensitivity of cytology against HPVmRNA test is 50% (95% CI 1.3%-98.7%). Specificity of cytology against HPV mRNA test is 90.6% (95% CI 75-98%). Positive predictive value of cytology against HPV mRNA test is 25.0% (95% CI 0.6-80.6%).

Negative predictive value of cytology against HPV mRNA test is 96.7% (95% CI 82.8-99.9%). Sensitivity of HPV mRNA test against histopathology is 100% (95% confidence interval 15.8%-100%). Specificity of HPV mRNA test against histopathology is 100% (95% confidence interval 89.1-100%). Positive predictive value of HPV mRNA test against histopathology is 100% (95% confidence interval 15.8%-100%). Negative predictive valueof HPV mRNA test against histopathology is 100% (95% confidence interval 89.1-100%).

Table 2: Association of colposcopic findings with CD4 cell count.

	Normal study (n=49)	Minor colposcopic findings (n=19)	Major colposcopic findings (n = 2)	X^2	P value
CD_4 cell count				36.90	0.002
< 200	1 (25%)	1 (25%)	2 (50%)		
200 – 500	24(64.9%)	13 (35.1%)	0		
> 500	24 (82.8%)	05 (17.2%)	0		

Table 3: Association of HPV mRNA test with colposcopy, cytology and histopathology.

	Positive (n=2)	Negative (n=32)	X^2	P value
Colposcopy			34.0	0.002
Normal	0	19 (59.3%)		
Minor	0	13 (40.6%)		
Major	2 (100%)	00		
Cytology				
NILM	0	29 (90.6%)		
ASCUS	01(50%)	03 (9.4%)	16.54	0.064
LSIL	01 (50%)	00		
Histopathology				
Positive	02 (100%)	0	34	0.002
Negative	0	32 (100%)		

DISCUSSION

Distribution of cases according to age

In the present study about 51% cases were from age group of 31-39 years. The two major colposcopic findings with HPV mRNA positive both were from the age group of 31-39 years. Out of 19cases with minor colposcopic changes, 12 cases (27.7%) were in the 31-39 years. A study by V Goel et al (2011), The median age was 30 years (range 21-43 years) with most of them 14, (35%) being in the age group 26-29 years followed by 13 (32%) between 30 and 35 years.[10] Similar study by Sarkar et al (2011), risk becomes higher when age was more than 30.[4] According to present study lower genital tract infections were more prevalent in age group between 31-39 years. Probably this was related with declining body immunity as seen in more advanced HIV infected cases.

Association of colposcopic findings with cytology

Findings suggested in this study that 91% cases revealed NILM, 7.1% revealed ASCUS and 1.5% revealed LSIL. 1 case with major colposcopic finding revealed LSIL and 1 case revealed ASCUS on pap smear reporting. This finding was found statistically significant (p=0.002).

Association of colposcopic findings with CD4 count

About 41% cases had CD4 >500 cells/cumm, 53% cases had CD4 200-499 cells/cumm and remaining 5.7% cases had CD4 cells<200 cells/cumm. There were 2 case with major colposcopic changes with positive HPV mRNA test had CD4 count was <200 cells/cumm. This finding was found to be statistically significant (Table 2).

In a study by Badkur et al, 4% of studied population had CD4 count of <200cells/mm, 36% had CD4 count of 200-499cells/cumm and 60% had CD4 cells >500cells/cumm and was statistically significant.[6] Similar results were reported by Harrish TG et al and in a study by S Rugpao et al, but these trends were not significant.[11,12]

Association of HPVmRNA testing with colposcopy

In the present study, HPV mRNA testing was done in 34 cases, of which 5.9% (n=2) cases were positive for high risk HPV infections whereas 32 out of 34 (94.1%) test reported negative for HPV mRNA test.

The test was not performed in 36 patients due to various logistic reasons (missing samples, leaked samples during transportation etc.).

Two cases with major colposcopic findings revealed positive HPV mRNA test and which was further confirmed by histopathology. This was statistically significant. (p=0.021) (Table 3). A prospective cohort study done by Sveinung Wergel and Sørbye et al, HPV mRNA positivity was 7.1% in women aged 25–33 years and 2.1% in women aged 34-69 years which was much lower than those reported from studies using HPV DNA tests.[13]

Association of HPV mRNA test with cytology

There were two patients with positive HPV mRNA test and in 1 case revealed ASCUS and in 1 case pap smear revealed LSIL. This finding was statistically significant (Table 3).

Association of HPV mRNA test with histopathology

There were two patients with positive HPV mRNA test i.e. overexpression of E6/E7 and in both cases histopathology detects the CIN2 and CIN3. This finding was statistically significant (Table 3).

CONCLUSION

In the present study, prevalence of lower genital tract infections in HIV positive women who were not on ART was 30.0%. In the present study, prevalence of high risk HPV was 5.9%.

Present study concludes that diagnostic efficacy of HPV mRNA test is similar to that of histopathology report. All HIV positive women not on ART should be made aware for importance of screening, early detection and management of lower genital tract infections, HPV infection CIN and cancer cervix.

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