

ORIGINAL RESEARCH

COMPARATIVE STUDY OF LIQUID-BASED CYTOLOGY VERSUS HPV DNA FOR SCREENING OF CERVICAL CANCER AT A TERTIARY HOSPITAL

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ABSTRACT

Background: Among Indian women, cervical cancer is the most common form of genital malignancy. Cytology (conventional or liquid-based) & HPV DNA used for diagnosis of cervical cancer. Present study was aimed to compare liquid-based cytology versus HPV DNA for screening of cervical cancer at a tertiary hospital.

Material and Methods: Present study was single-center, prospective, observational study, conducted women between the ages of 21 - 65 years, with white discharge per vaginum, post coital bleeding or irregular bleeding, unhealthy cervix on speculum examination.

Results: In present study, 220 women were evaluated for LBC & HPV DNA testing. Mean age of women was 42.91 ± 6.19 year, mean age at marriage was 17.41 ± 3.63 years & mean duration of sexual life was 26.81 ± 8.63 years. Majority were from lower socioeconomic status (57.27 %). Common chief-complaint was white PV discharge (64.55 %), Post coital bleeding PV (19.55 %) & intermenstrual bleeding PV (15.91 %). On per-speculum examination cervical erosion (40.45 %) was common finding. Common mode of contraception used was tubectomy (49.55 %), while 28.64 % women were not using any contraceptive. In present study, common LBC findings were Inflammatory / reactive changes (48.18 %), Non-specific inflammation (10.91 %), Candida (5.45 %), Trichomonas vaginalis (3.64 %), Bacterial vaginosis (0.91 %), Atrophy (1.36 %). Pathological findings were ASCUS (5.00 %), ASC-H (0.91 %), LSIL (0.45 %), HSIL (0.91 %), HSIL with suspicious of invasion (0.45 %) & Squamous Cell Carcinoma (2.27 %). Normal findings were noted in 18.64 % women while 2 smears were labelled as unsatisfactory smear. HPV DNA was positive in 15.45 % women. HPV DNA was significantly associated with pathological LBC report (low grade, high grade & neoplasia).

Conclusion: HPV testing in comparison to LBC was more effective, but it is costlier for application as population screening tool.

Keywords: LBC, HPV DNA, cervical cancer, screening.

INTRODUCTION

Among Indian women, cervical cancer is the most common form of genital malignancy. While worldwide it is second only to breast cancer in incidence and approximately three-fourths of cases occur in the developing countries.^[1,2] Cervical cancer is a preventable disease. Prevention lies mainly in early detection. The risk of cervical cancer is mainly due to persistent HPV infection, older age, and other demographic, behavioural, and medical risk factors, that vary among population worldwide.^[3]

WHO currently recommends 3 screening tests for HPV: 1) Nucleic acid amplification tests (NAAT) for hr-HPV types (hr-HPV DNA/NAAT and mRNA), 2) Visual inspection with acetic acid or with Lugol's iodine (VIA/ VILI) by naked eye or magnified by colposcope or camera & 3) Cytology (Conventional Pap/Liquid-based cytology/Dual staining to identify p16 and Ki-67).^[4]

Cytology (conventional or liquid-based) is the most commonly used test that has been shown to dramatically reduce cervical cancer incidence and cancer-related deaths worldwide.^[5] Scientific evidence for the diagnostic and preventive value of the human papillomavirus (HPV) test has also been established.^[6,7] Present study was aimed to compare liquid-based cytology versus HPV DNA for screening of cervical cancer at a tertiary hospital.

MATERIAL AND METHODS

Present study was single-center, prospective, observational study, conducted in Department of Obstetrics & Gynaecology, Government Medical College, Doda, India. Study duration was of 1 year (January 2021 to December 2021). Study was approved by institutional ethical committee.

Inclusion criteria

- Women between the ages of 21 - 65 years, with white discharge per vaginum, post coital bleeding or irregular bleeding, unhealthy cervix on speculum examination, willing to participate & follow-up.

Exclusion criteria

- Women who had undergone hysterectomy with removal of cervix,
- Women underwent treatment for cervical carcinoma/ premalignant lesions of cervix
- Pregnant women
- Patients with massive bleeding per vaginum,
- Non-co-operative patients,
- Patients not willing to participate

Study was explained to women & a written informed consent was taken. Detailed history, physical examination findings were noted. Specimens were obtained for Liquid based cytology, as endocervical brush was inserted into the endocervical canal and rotated 360° 3-4 times. Brush was detached and placed into a vial containing fixative for transport composed

of 10% formalin 50ml, sodium chloride 0.5gm, sodium citrate 0.5gm and isopropyl alcohol 50ml.

The vial is then closed and shaken to obtain a homogenous mixing. The vial mixture was centrifuged at 2000 rpm for 10 minutes. The supernatant was discarded. To the cell pellet at the base of the tube, 1.5 ml of polymer solution was added. This mixture was once again mixed with vortex and centrifuged. With the help of the micropipette 50 ml of the suspensions was taken and placed over the slide in a circular manner. The slides are dried and stained with conventional pap stain.

HPV DNA testing was done on the residual cytobrush sample. DNA was extracted from the cytobrush sample and conventional PCR was carried out for the detection of HPV common and HPV 18 E6 and E7 oncoproteins.

Data was collected and compiled using Microsoft Excel, analysed using SPSS 23.0 version. Frequency, percentage, means and standard deviations (SD) was calculated for the continuous variables, while ratios and proportions were calculated for the categorical variables. Difference of proportions between qualitative variables was tested using chi-square test or Fisher exact test as applicable. P value less than 0.5 was considered as statistically significant.

RESULTS

In present study, 220 women were evaluated for LBC & HPV DNA testing. Mean age of women was 42.91 ± 6.19 year, mean age at marriage was 17.41 ± 3.63 years & mean duration of sexual life was 26.81 ± 8.63 years. Majority were from lower socioeconomic status (57.27 %). Common chief-complaint was white PV discharge (64.55 %), Post coital bleeding PV (19.55 %) & intermenstrual bleeding PV (15.91 %). On per-speculum examination cervical erosion (40.45 %) was common finding. Common mode of contraception used was tubectomy (49.55 %), while 28.64 % women were not using any contraceptive.

Table 1: General characteristics

Characteristic	No of women (n=220)	Percentage
Age (in years)	42.91 ± 6.19	
Age at marriage (in years)	17.41 ± 3.63	
Years of sexual life (in years)	26.81 ± 8.63	
Socioeconomic status		
Lower	126	57.27%
Middle	78	35.45%
Upper	16	7.27%
Chief-complaint		
White discharge PV	142	64.55%
Post coital bleeding PV	43	19.55%
Intermenstrual bleeding PV	35	15.91%
Per-speculum findings		0.00%
Cervical erosion	89	40.45%
Normal	131	59.55%

Contraception		
Tubectomy	109	49.55%
None	63	28.64%
Other	28	12.73%
Barrier	11	5.00%
OCP	9	4.09%

In present study, common LBC findings were Inflammatory / reactive changes (48.18 %), Non-specific inflammation (10.91 %), Candida (5.45 %), Trichomonas vaginalis (3.64 %), Bacterial vaginosis (0.91 %), Atrophy (1.36 %). Pathological findings were ASCUS (5.00 %), ASC-H (0.91 %), LSIL (0.45 %), HSIL (0.91 %), HSIL with suspicious of invasion (0.45 %) & Squamous Cell Carcinoma (2.27 %). Normal findings were noted in 18.64 % women while 2 smears were labelled as unsatisfactory smear. HPV DNA was positive in 15.45 % women.

Table 2: LBC & HPV DNA findings

Findings	No. of women	Percentage
LBC findings		
Normal	41	18.64%
Non-specific inflammation	24	10.91%
Inflammatory / reactive changes	106	48.18%
Atrophy	3	1.36%
Trichomonas vaginalis	8	3.64%
Candida	12	5.45%
Bacterial vaginosis	2	0.91%
ASCUS	11	5.00%
ASC-H	2	0.91%
LSIL	1	0.45%
HSIL	2	0.91%
HSIL with suspicious of invasion	1	0.45%
Squamous Cell Carcinoma	5	2.27%
Unsatisfactory smear	2	0.91%
HPV DNA		
hrHPV DNA positive	34	15.45%
hrHPV DNA negative	186	84.55%

ASCUS - Atypical squamous cells of undetermined significance, ASC - Atypical squamous cells – high grade, LSIL - Low grade squamous intraepithelial lesions, HSIL - high grade squamous intraepithelial lesions,

HPV DNA was significantly associated with pathological LBC report (low grade, high grade & neoplasia).

Table 4: Association of LBC and HPV

LBC Report	Number of HPV positive cases	Number of HPV negative cases	P value
Normal/Inflammatory/other	17	180	<.0001
Low grade	9	5	<.0001
High grade	3	1	<.0001
Neoplasia	5	0	<.0001

DISCUSSION

The pathogenesis of cervical cancer is associated with human papillomavirus (HPV) infections and consists of several steps involving cell proliferation outside the human body's control mechanisms. This process results in a cascade of malignantly transformed cells in the following order: hyperplasia, dysplasia, carcinoma in situ, and invasive carcinoma.^[8,9]

High parity, smoking, nutrition and use of combined hormonal oral contraceptives for more than 5 years have been reported as major environmental risk factors for cervical cancer in various studies.^[10] Infection with other sexually transmitted diseases such as HIV, herpes, chlamydia, gonorrhea and syphilis increases the cervical cancer risk.^[11] Universal cervical cancer screening in India remains an unmet need. According to the National Family Health Survey (NFHS-4), only 22.3% of eligible women received cervical cancer screening during 2015–2016.^[12]

Liquid based cytology (LBC) is the most accepted method for detection of premalignant lesion and improves the smear sensitivity. The advantage of LBC include removal of blood and mucus, obscuring cells, reduction of unsatisfactory smears and provision of cells for detection of HPV, presence of residual sample for performing ancillary techniques such as immunocytochemistry.^[13] Screening approaches that use HPV-DNA testing may prove more practical when incorporated into strategies less dependent on existing laboratory infrastructure and requiring fewer visits.^[14,15]

Detection of high-risk HPV does not always mean that there is cancer or its precursor; it simply shows that there is an HPV infection. In a woman aged 35 years or older, HPV/DNA test performs better than in younger women, as a positive HPV/DNA test is more likely due to a persistent HPV infection. The average sensitivity & specificity in this age cohort are 89% and 90% respectively.^[16] A randomized trial in Osmanabad district in India demonstrated a single round of HPV-DNA testing among enrolled women between the ages of 30 and 59 years, was associated with a significant reduction in the number of advanced cancers and deaths.^[17]

In study by Linda AL et al,^[18] among 2,627 screened women, cytological sensitivities (Pap, LBC: 47%) were lower than HC2 (95%) and PCR (79%) for CIN2p. Co-testing demonstrated higher sensitivities (HC2 co-testing: 99%; PCR co-testing: 84%), but at the cost of lower specificities (92%–95%) compared with HPV stand-alone (HC2:95%; PCR: 94%) and cytology (97% or 99%). Co-testing versus HPV stand-alone showed equivalent relative sensitivity [HC2:1.06, 95% confidence interval (CI), 1.00–1.21; PCR: 1.07, 95% CI, and 1.00–1.27]. Relative specificity of Pap cotesting with either HPV test was inferior to stand-

alone HPV. LBC co-testing demonstrated equivalent specificity (both tests: 0.99, 95% CI, and 0.99–1.00).

In study by Perera KCM et al,^[19] 25 (6.36%) women among 35 and 18 (4.61%) among 45-year-old women were positive for HPV/DNA test. The number of 7 (1.78%) among 35 year and 5 (1.28%) among 45 year old women had \geq ASCUS in conventional cytology, while the number of 10 (2.54%) among 35 years and 8 (2.05%) among 45 years old women had \geq ASCUS in LBC. Prevalence of CIN by colposcopy among 35 years and 45 years women for LBC vs conventional cytology were 1.53%, 1.28% and 1.53%, 1.03% respectively and there was no significant difference. No invalid results were reported for LBC and the treatment adherence for colposcopy was 86.7%.

Manga MM et al,^[20] noted that among 209 participants, cytological findings were normal in 126 (61.6%) women while 80 (39.0%) had abnormal features. Three (1.4%) respondents had unsatisfactory smears. The observed abnormal cytological features include HPV changes 30 (14.4%), HPV changes with inflammation 2 (1.0%), inflammatory changes alone 36 (17.3%), Low Squamous Intraepithelial Lesion; LSIL 3 (1.4%), High Squamous Intraepithelial Lesion; HSIL 5 (2.4%) and malignant changes 3 (1.4%). Positive HPV DNA testing was detected among 100 (48.1%) of the participants. Almost half 60 (47.6%) of the women with normal cytology were positive for HPV. Among women with cytologically detected HPV changes, only 16 (50%) were also HPV DNA positive. The sensitivity and specificity of cervical cytology in detecting HPV infection was 16.2% and 85.0% respectively.

Raj S et al,^[21] studied 506 women, mean age of patients in the study was 43.36 years. The sensitivity and specificity of LBC was 76.47%, 43.85%, and that of HPV DNA was 88.23%, 57.89%. The co-testing of LBC and HPV-DNA had a sensitivity and specificity of 94.11% and 24.56%. Discriminatory power of LBC (AUC 0.6; 95% CI: 0.48 to 0.71) and HPV DNA (AUC 0.73; 95% CI: 0.61 to 0.83) was acceptable. Among all the parameters, HPV DNA was the best predictor of pre-invasive or invasive lesion with 73.00% chances of correctly predicting pre-invasive or invasive lesion. Though the early detection and treatment of pre-invasive and invasive lesion of cervix is required, it is important that the best and most sensitive diagnostic tools are used for the screening purposes. Overall HPV DNA was best predictor of pre-invasive or invasive lesion with significantly higher diagnostic accuracy as compared to co-testing. Similar findings were noted in present study.

The shift from conventional cytology to liquid-based cytology in the screening of cervical lesions would result in improving the sample quality, reproducibility, sensitivity, specificity as well as the ability to perform molecular testing.^[22] However, cytology based cervical screening also has some limitations. The major problem is the low sensitivity of a single smear to detect high grade precursor lesions (50%–70%), which require frequent testing.^[23] In addition; cytology has low reproducibility, leading to variable accuracy.^[24]

Screening is a secondary level of prevention which is done to detect or rule out disorders at an early stage in healthy person. Screening and treatment of Precancerous lesion of cervical cancer is one of the secondary prevention activities. LBC with concomitant HPV testing is more effective & recommended,^[21] but it is a costly investigation ideally suited for high-resource setting.

CONCLUSION

HPV testing in comparison to LBC was more effective, but it is costlier for application as population screening tool. LBC is better alternative to conventional smear because of lower rate of unsatisfactory smears. Furthermore, residual LBC sample is available to perform HPV DNA testing.

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