

ORIGINAL ARTICLE

Comparison of Liquid Based Cytology (LBC), Conventional Pap Smear and Human Papilloma Virus Detection (When indicated) to Detect Cervical Premalignant and Malignant lesions

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Abstract:

Background: There is a strong causal association between human papillomavirus (HPV) and cervical cancer. Currently HPV DNA test is recommended as a triage for minor Cytological abnormalities and follow up of high-grade lesions. **Aims:** To examine the efficacy of Conventional Pap smear (CPS), Liquid based cytology (LBC) and HPV (Human papilloma virus) DNA test as independent screening tests to detect squamous intraepithelial lesions (SIL) of the uterine cervix. To study the efficacy of dual screening tests by using the following combinations: a) CPS and LBC, b) CPS and HPV DNA test, c) LBC and HPV DNA test with colposcopy as the gold standard. **Material and Methods:** This is a cross sectional screening study inclusive of 1064 women. Either CPS smears alone or both CPS and LBC were prepared. HPV DNA testing was done for cases reported as atypical squamous cells of undetermined significance (ASC-US) or more on cytology. Statistics-Cohen's Kappa coefficient is a statistical measure of agreement of the screening test with the gold standard. **Results:** We found maximum Sensitivity (Sn) with LBC (89.66%) as a stand-alone test. While in dual screening testing, maximum Sn was obtained by combining CPS with LBC. Maximum Specificity (Sp) was obtained with HPV DNA test (97.89%) when done independently and it was of high diagnostic value (Sp=100%) when done in combination with either CPS or LBC. **Conclusion:** The combination of HPV DNA test with either CP or LBC had best agreement with the Colposcopy when done as a triage for borderline cytological abnormalities.

Keywords-

LBC, CPS, HPV DNA, Colposcopy, Sensitivity, Specificity introduction

Introduction:

Cancer cervix is the fourth most common cancer worldwide. According to latest WHO statistics 2018, world incidence of

cervical cancer is approximately 5, 69,847 new cases accounting for 6.9% of all cancers in women. India alone accounts for one-quarter of the worldwide burden of cervical cancer cases. It is one of the leading causes of cancer mortality, accounting for 17% of all cancer deaths among women aged between 30 and 69 years. Early detection by screening all women for cervical lesion has a very crucial role in prevention of cancer cervix. There is strong causal association between cervical cancer and HPV infection. Currently the options available for screening are the VIA (Visual inspection of acetowhite areas), Conventional Pap smear (CPS), Liquid based Cytology (LBC) and Human papilloma virus (HPV) DNA test. Currently HPV DNA test is recommended as a triage for minor cytological abnormalities and follow up of high-grade lesions after treatment. On evaluation of several cross-sectional studies it is found that the pooled sensitivity of HPV test for detection of cervical intraepithelial neoplastic (CIN) 2+ and 3+ diseases was 90 and 95 % respectively. Randomized controlled trials demonstrated higher detection rates of CIN 2+ and 3+ by HPV test than cytology. However, the Specificity of HPV DNA test is lower as compared to that of cytology. This study was undertaken with the hypothesis that dual screening using cytology and HPV DNA test is better than cytology and HPV DNA test when used alone. The primary objective of this study was to examine the efficacy of CPS, LBC and HPV DNA test as independent screening tests to detect squamous intraepithelial lesions (SIL) of uterine cervix. The secondary objectives were to study the efficacy of dual screening tests by using the following combinations: a) CPS and LBC, b) CPS and HPV DNA test, c) LBC and HPV DNA test with colposcopy as the gold standard. We also compared the cytomorphological features as seen in CPS and in LBC smears.

Material and Methods:

A cross sectional screening study was conducted over a period of 2 years. A total number of 1064 sexually active women between the age group of 15-70 years were included in the study and the women with active per vaginal bleeding due to any reason and pregnant women

were excluded. Written informed consent was taken after explaining the procedure to the patients. All information and details about signs and symptoms were filled out for each patient in a detailed proforma. Permission for the study was sought from the institutional ethics committee. Either CPS alone (using Ayer's spatula) or both CPS and LBC (split samples using Cervix brush) were prepared from women attending various community camps and gynecology outpatient departments at tertiary care hospital. A smear was made on a glass slide for CPS and wet fixed. For split samples, after preparing a CPS, the head of the cervix brush was dropped in a vial containing 10ml preservative fluid for LBC and the same was used for HPV DNA testing. CPS and LBC reporting was done by a single observer. Cytology was positive when samples showed epithelial Abnormalities as Atypical Squamous cells of undetermined significance (ASC-US) and more. HPV DNA testing was done by Hybrid capture II technique for cases reported as ASC-US or more. Cytology was reported using Bethesda system 2014. Women positive with any of the 3 tests (CPS, LBC & HPV DNA) were advised to undergo the standard procedure of Colposcopic evaluation and directed biopsy.⁷ The various measures of the accuracy of CPS, LBC, HPV DNA detection tests as a single screening test; and their various combinations in dual screening were calculated using Colposcopy as the gold standard. The ASC-US, Atypical Squamous cells of undetermined significance cannot exclude high grade SIL(ASC-H), Low grade squamous intraepithelial lesion (LSIL), High grade squamous intraepithelial lesion (HSIL), Atypical glandular cells (AGC) and all malignancies reported on CPS or LBC were considered as positive results. The Colposcopy with aceto-white lesions was considered as the positive test. For HPV DNA test ratio >0.80 was considered as positive. Cohen's Kappa (K) coefficient is a statistical measure of agreement of the screening test with the gold standard. Value of K <0.20 is interpreted as poor and the value of K 0.81- 1.00 is interpreted as very good.

Results:

Hierarchy chart (Fig 1) is showing distribution of total 1064 cases studied. According to chart 1 The total number of CPS smears and LBC specimens collected were 903 and 902 respectively. Both CP and LBC (dual screening) was performed in 741 cases. Only LBC was performed in 162 cases and only CP was performed in 161 cases as a stand alone test for initial screening. Colposcopy was done in 271 cases. HPV DNA detection test was performed in 195 cases. Histopathological examination and immunocytochemistry was performed as and when required. In the present study, out of total 903 CPS, 794 (87.93%) were satisfactory for evaluation and 109 (12.07%) were unsatisfactory due to obscuring of cells by mucus, blood, debris, inflammation or scanty cellularity.

Out of a total 902 cases of LBC, 848(94.02%) were for similar reasons. The rate of satisfactory smears improved with LBC by 7%. The adequacy rate (representation of glandular cells or metaplastic cells in the smear) also improved with LBC by 3.76%. In the present study the most common organism detected was Candida (CPS -4.87% and LBC-5.32%), followed by Bacterial vaginosis (CPS-1.99%, and LBC- 2.32%), Trichomonas vaginalis (CPS-0.33% and LBC- 0.66%), Actinomyces (CPS-0.11% and LBC- 0.22%). Overall the rate of detection of various organisms was found to be higher by LBC. As shown in figure 2 the total number of SILs detected by CPS was 85 (11.48%) and by LBC were 96 (12.97%). Thus the rate of positivity of LBC was found to be 1.49% higher than CP. With the CPS, the various epithelial abnormalities detected were as follows: ASC-US (83) followed by LSIL (22) and HSIL (13). 3 cases were reported as squamous cell carcinoma. With the LBC the various epithelial abnormalities detected were: ASC-US (93) followed by LSIL (25) and HSIL (8). 2 cases were reported as squamous cell carcinoma and a single case was reported as another category- rhabdomyosarcoma both in CP and LBC. Table 1 shows that the detection rate of ASC-US (6.6% vs. 8.36%) and LSIL (2.56% vs. 2.83%) was higher by LBC when split samples were prepared from material collected on the cervix brush. However ASC-H and HSIL and AGC were detected better by CPS. Squamous cell carcinoma and other malignancies were equally detected by both the tests. Accuracy parameters that were studied are shown in Table 2. As per table 2, maximum Sensitivity was obtained by LBC (89.66%) as a stand-alone test. While in case of dual screening testing, maximum sensitivity was obtained by combining CPS with LBC. Maximum Specificity was obtained with HPV DNA test (97.89%) when done independently and it was of high diagnostic value (Sp=100%) when done in combination with either CPS or LBC. There was maximum PPV obtained by combination of HPV DNA test with either CPS or LBC (100%). It means that if the HPV DNA test is negative, there are very rare or no chances that a woman will have a precancerous lesion. NPV were maximum by the combined screening with LBC and HPV DNA test (97.22%). Both positive and negative likelihood ratios were found to be highest with the HPV DNA test when it was done independently (25.41%). With the dual screening strategy, the test qualities appear to be better as compared to the use of a single screening test. However, the P value was found to be non-significant for all the 3 combinations of the screening tests. When we compared the cytomorphology as seen in CPS and LBC, it was found that, in CPS smear, there was more cellular overlap, air drying artifacts and obscuring of cells by mucus, blood and inflammatory cells. While the cellular architecture was like glandular sheets, clusters were better preserved as compared to LBC. In LBC smears, cells were

more uniformly dispersed with minimal overlap. In LBC due to better fixation of cells, air-drying artifacts^{8,9,10} were absent. Cell morphology was well preserved in both of the techniques.

Figure 1: Hierarchy Showing Distribution Of Cases (n= 1064)

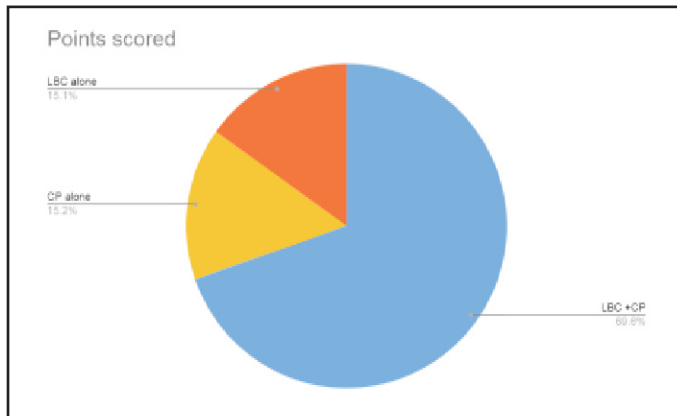


Table1: Detection Rate of Epithelial cell abnormalities in Split Samples processed by CPS and LBC (N=741)

Epithelial lesions	CP (n=741)		LBC (n=741)	
	No.	Percentage	No.	Percentage
ASC-US	49	6.6%	62	8.36%
ASC-H	04	0.53%	02	0.26%
LSIL	19	2.56%	21	2.83%
HSIL	08	1.07%	07	0.94%
SCC	02	0.26%	02	0.26%
AGC	02	0.26%	01	0.13%
Other-rhabdomyosarcoma	00	0.13%	01	0.13%
Total	85	11.47%	96	12.95%

(Sn- sensitivity, Sp- specificity, PPV- positive predictive value, NPV- negative predictive value, LR+ - likelihood ratio positive, LR- - likelihood ratio negative)

Discussion :

Cervical cytology plays an extremely important role in prevention of cancer cervix by detecting premalignant and malignant lesions of the cervix. The HPV DNA test is

Figure 2: LBC smear eliciting High grade Squamous Intra epithelial Lesion (400x)

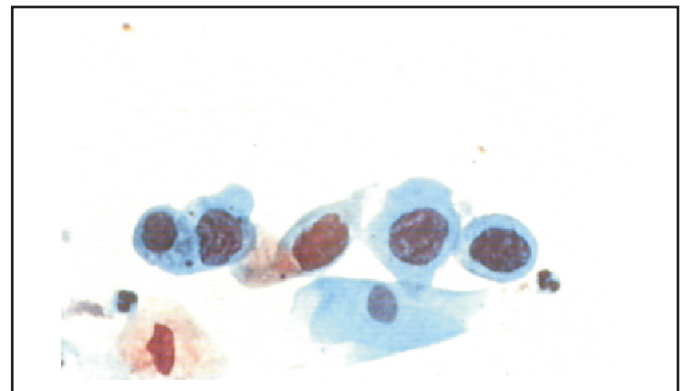


Table2: Comparison of Test Qualities of Various Tests Done Either Singly or In Combination with Other Test (Dual Screening)

	Screening by Single test			Dual Screening		
	CPS	LBC	HPV	CPS +LBC	CPS +HPV	LBC + HPV
Sn	84.85%	89.66%	53.49%	89.29%	87.5%	85.7%
Sp	86.76%	86.21%	97.89%	87.06%	100%	100%
PPV	67.47%	68.42%	82.30%	69.44%	100%	100%
NPV	94.65%	96.15%	84.06%	96.10%	96.34%	97.22%
LR +	6.411	6.5	25.41	6.89	undefined	Undefined
LR -	0.1746	0.12	0.4751	0.1231	0.125	0.1429
Cohen's kappa	0.6587	0.6875	0.5803	0.6967	0.9148	0.9091
P value	0.0085	0.0388	0.00028897	NS	NS	NS

another option being considered as a primary screening test. Researchers are going on to know which screening test

Table 3: Comparison of sensitivity and specificity of CP and LBC with other studies when used as a single test.

Studies	CP		LBC	
	Sensitivity	Specificity	Sensitivity	Specificity
Sheets et al ¹⁶ (1995)	67.3%	76.9%	73.6%	76.2%
Sylvia Taylor et al (2006) ¹⁷	78.6%	86.7%	73.0%	86.5%
H. Beerman et al (2012) ¹⁸	92.04%	98.17%	96.24%	97.75%
Sherwani et al (2014) ¹⁹	53.7%	50%	97.6%	50%
Present study	84.85%	86.76%	89.66%	86.21%

will provide maximum screening benefits in combination or singly. In some screening programs HPV test and cytology (co-testing) were done simultaneously, and women positive on either test were referred for colposcopy. In a study 1064, women (=30 years) were screened by both HPV test and conventional cytology (co-testing).¹¹ Women negative on both tests were rescreened after 3 years. The 5-year risk of invasive cervical cancer of women negative on co testing was low (3.2/100,000/year) and was almost similar (3.8/100,000/year) to the women negative done independently (25.41%) (Table3). Despite this fact, in a developing country like ours, HPV DNA test cannot be used as the stand alone screening test due to its cost factor. Also most women with high risk HPV DNA positive results and normal Pap test results have transient HPV infections (80%) that will not progress to high grade cervical disease or cancer. When the screening tests were done in combinations (Table 3), the CP+LBC combination had the best ability (Sn 89.29%) to detect the squamous intraepithelial lesion (SIL). While the ability to rule out the squamous intraepithelial lesion was found to be best when HPV DNA test was combined with either CP or LBC (Sp 100%). The maximum agreement (Cohen's kappa) with the Colposcopy was obtained by the combination of HPV it requires initial training when there is switch over from CPS specially in the interpretation of atrophic smears and immature metaplastic cells. The classical tumor diathesis

Table 4: Comparison of Rate of Epithelial Abnormalities Detected By CP and LBC

	H. Beerman et al (2012) ¹⁸		M. TuncCandae t al (2009) ²⁰		HaoDeshou et al(2009) ²¹		M. Jorg Obwegeser et al(2001) ²²		Sherwani RK et al 2014 ¹⁹		Present study	
	CP	LBC	CP	LBC	CP	LBC	CP	LBC	CP	LBC	CP	LBC
ASCUS	0.84%	2.07%	0.5%	3.12%	2.30%	6.82%	1.4%	1%	-	-	6.6%	8.36%
ASC-H			-	-	0.06%	0.31%		-	-	-	0.53%	0.26%
LSIL	0.22%	0.27%	1.4%	2.25%	0.02%	0.04%	3.7%	4.7%	10.6%	18.2%	2.56%	2.83%
HSIL	0.64%	0.56%	0.20%	0.25%	0.28%	1.80%	1.8%	1.6%	0.6%	4.3%	1.07%	0.94%
Malignancy	0.006%	0.008%	-	-	0.08%	0.2%	0.1%	0%	3.7%	3.7%	0.39%	0.39%
AGC	-		-	-	-	-	-	-	-	-	0.26%	0.13%

DNA test with either CP (0.90) or LBC (0.91). With all the 3 dual screening strategies, the test qualities appear to be better as compared to the use of a single screening test, however the p value was found to be non-significant for all the 3 combinations of the screening tests. This indicates that the improvement in the test parameters of dual tests may have been by chance. So beginning with LBC or CPS as a screening test and only getting an HPV DNA detection test if the cytology results are borderline; might be the best option. The borderline lesions like ASC-US and ASC-H are most difficult to detect and diagnose confidently with the limitations of the CPS. In the 13 mm diameter of monolayer LBC preparations, the pickup rate of ASC-US was significantly better in the present study (ASC-US 8.36% by LBC vs 6.6% by CP). The randomized representative sample transferred on to the glass slide helps to achieve a better pick up rate. The observation of the present study was in concordance with that of other studies in the literature (Table 4). 18,19,20 Detecting more number of ASC-US is important because 7.13% of these progresses to high-grade lesions. For the rest of the epithelial abnormalities, the rate of detection was almost similar by both CPS and LBC. And these rates were also comparable with other studies (Table 4). Also, a lesser unsatisfactory rate with LBC smears of 5.98% as compared to 12.07% by CPS was comparable with other studies. In the present study, the adequacy rate, meaning that there is representation of the TZ components, was improved by 5% with LBC as compared to CP. The limitations of LBC is that of squamous cell carcinoma, seen in the CPS, is not as well appreciated in the LBC smear and requires regular experience. However, LBC has definite advantages like the ease of reflex HPV DNA testing and preparing multiple smears (when need be) from the cell pellet left behind in the centrifugation tube. These additional smears can be of

great use for immunocytochemistry, and we did apply markers like P16, ink4, Ki67 when needed. Some of the future Recommendations which we would like to put forth from our study are: It appears that there is a marginal improvement in the test qualities with LBC either as a stand-alone test or in combination with CPS or HPV DNA test. Therefore, the CPS may be continued as the primary screening test in resource poor settings. We strongly recommend the triaging of CPS with HPV DNA test for high risk viruses in all women who have epithelial abnormalities. Although we are adding a costly test in the screening programme, this is helping in identifying those women who have high chances of progressing to invasive cancers. These women can be benefited by regular follow-up. If resources are available to initiate and sustain the screening of all women with LBC then it may be introduced as the primary screening test. Another prerequisite of adopting LBC as the screening test will be training of screeners to avoid over diagnosis of squamous intraepithelial lesions (SIL).

Conclusion:

With the stand alone test strategy, the efficacy of LBC to detect the squamous intraepithelial lesion of the cervix was better than CP and HPV DNA test. HPV DNA test independently had very good specificity. Of the 3 tests CP, LBC and HPV DNA test, LBC had the best agreement with the Colposcopy followed by CP and HPV DNA test. With the dual screening strategy, the combination of CP+LBC had the best ability to detect the squamous intraepithelial lesions (SIL) of the uterine cervix. HPV DNA test had better NPV thus can improve the cervical cancer screening interval if the test is negative. The combination of HPV DNA test with either CP or LBC had best agreement with the Colposcopy when done as a triage for borderline cytological abnormalities

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