

## Original Research Article

# Comparative study of conventional Pap smear and liquid based cytology as a screening method for cervical cancer

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## ABSTRACT

**Background:** Pap smear is the conventional screening procedure for cervical cancer. Liquid based cytology has been developed as a cost effective alternative as it has a short screening time, better morphology and clean background while also providing residual material to test for HPV DNA. Therefore this study is undertaken to know the role of Liquid-based cytology in evaluating pre-malignant and malignant lesions of cervix. Objectives of current study were to study the distribution of various cervical lesions on liquid-based cytology and compare them with conventional Pap smears. To know the role of liquid-based cytology in evaluating pre-malignant and malignant lesions of cervix

**Methods:** This study included 200 women attending to gynaecology OPD and the samples were taken for both conventional cytology and liquid based cytology. The smears were studied in detail and were interpreted as per The Bethesda system 2014 of reporting pap smears and results are recorded and compared.

**Results:** The commonest cervical lesions on pap smears by liquid-based cytology are NILM-reactive changes (N=92, 46%), NILM Candida infection is seen in 9 cases (4.5%) and trichomonas vaginalis infection in 5 cases (2.5%). Unsatisfactory smears on LBC is less when compared to conventional smears as the coefficient of correlation is significant with p value of 0.000422 (<0.05). The number of cases with a diagnosis of ASCUS is reported more in liquid-based cytology (9 cases) when compared to conventional Pap (8 cases). The number of cases with diagnosis of HSIL, SCC is reported more in liquid based cytology (7 cases) when compared to conventional Pap (4 cases). Epithelial cell abnormality were easily diagnosed on LBC smears with significant p value of 0.002414 (<0.05).

**Conclusions:** Liquid-based cytology has advantages of fewer unsatisfactory smears and better detection of epithelial cell abnormalities when compared to conventional Pap smears. LBC is better for the screening of premalignant and malignant lesions of cervix even though it is costly.

**Keywords:** Conventional Pap smears, Comparative study, Epithelial cell abnormality, Liquid based cytology

## INTRODUCTION

Cervical cancer is a major public health problem worldwide and needs attention and screening. conventional Pap smear is routinely used to screen cervical premalignant and malignant lesions but has many disadvantages and limitations. In the present era, liquid-based cytology is costly but has certain

advantages. The technique improves specimen adequacy and detection of high grade intraepithelial lesions.<sup>1</sup>The developed countries changed their cervical screening method from Pap test to Liquid-based cytology in 2008.<sup>2</sup> The FDA for cervicovaginal testing has defined two systems in Liquid-based cytology: sure path (by BD) and Thin prep (by Hologic). The sample is collected in a conventional manner and instead of transferring material

on the slide, it was transferred to a vial of transport medium. Advance investigations like immunohistochemistry, and HPV detection can be done from the sample besides cytological examination. In the sure path method, the sample is vortexed, strained, and centrifuged. The cells form a circle of 12.5 mm in diameter. In the Thin prep method, the cells are collected on the surface of the filter when the vacuum is applied. The filter is then pressed against a slide to transfer the cells into a 20 mm diameter circle.<sup>3</sup>

In both the methods we get well preserved monolayered cells in the background devoid of blood and mucus. The high cost of the instrument and the requirement of a trained cytopathologist are some of the limitations in adopting this method.<sup>4</sup> Significant advances in screening have lowered mortality in developed countries. 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. This study was conducted to know the accuracy of liquid-based cytology in evaluating pre-malignant and malignant lesions of cervix and compare them with conventional Pap smears.

## METHODS

### *Study design, location duration and sample size*

Current study was an observational study conducted at Andhra medical college, Visakhapatnam, Andhra Pradesh for two years from November 2018 to October 2020 at department of pathology and obstetrics and gynaecology on 200 patients.

### *Inclusion criteria*

Inclusion criteria for current study was patients above 30 years of age attending to gynaecology OPD with complaints of white discharge, bleeding per vagina, irregular menses, pain in lower abdomen, post-coital bleeding, excessive purulent discharge, postmenopausal bleeding are included.

### *Exclusion criteria*

Hysterectomised women, women with frank growth, women who have undergone prior treatment for cancer cervix are excluded.

### *Methodology*

The clinical data was recorded with per speculum and per vaginal findings. Consecutive samples were taken and limited to 200 cases as liquid based cytology is costly test. Ayre's spatula was introduced into the external cervical os and cells were collected from ectocervix and endocervix. A smear was made on a glass slide for conventional pap smear (CPS) and fixed in 90% Iso

propyl alcohol for 30 minutes. Then for Liquid based cytology a cytobrush was introduced into the external os and scraped to collect cells from ectocervix and endocervix. The cytobrush was detached and dropped in a vial containing the preservative fluid for liquid based cytology. The method adopted was sure path method: Took 4 ml of separator solution in a centrifuge tube and added the sample slowly into the tube through the walls so that a clear cut demarcation should be visible between the sample and separator solution. Centrifuged the tube for 2000 rpm for 5 minutes. Discarded the supernatant layer by dropper leaving the pellet undisturbed. Vortex the pellet to make sure that the pellet has been homogenated and then added 2 drops of normal saline/distilled water to the pellet. Now the cells were ready to be prepared into smear. Empty slide with cyto cylinder was kept ready. The space in the cylinder was made wet with 2 drops of distilled water. 50 microliters of pellet was introduced into the cylinder through the micropipette. Kept the prepared slide with cylinder into the nanocyto centrifuge, set for 1200 rpm for 5 minutes. After 5 minutes, carefully removed the cyto cylinder from the slide and immediately transferred the slide into isopropyl alcohol. Both conventional Pap smears and smears from liquid based cytology were stained with Pap stain. The smears were studied in detail and were interpreted as per The Bethesda system 2014 of reporting pap smears. The results were recorded and compared.

### *Statistical analysis*

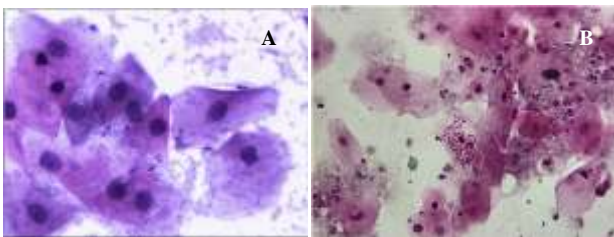
The percentages and coefficient of correlation were calculated using SPSS version 24.

## RESULTS

In the present study analysed 200 Pap smears on liquid-based cytology and compared the results with conventional Pap smears. The commonest cervical lesions on Pap smears by Liquid-based cytology are NILM-reactive changes (N=92, 46%), followed by NILM-normal (N=43, 21.5%) and NILM-non specific inflammation (N=27, 13.5%), NILM-Candida infection is seen in 9 cases (4.5%) (Figure 1) and Trichomonas vaginalis infection in 5 cases (2.5%). Epithelial cell abnormality was seen in 17 cases (8.5%). Squamous cell carcinoma in 4 cases (2%). Unsatisfactory smear is seen in 1% of cases (Table 1).

NILM was more common in < 45 years (N=147, 73.5%), ≥45 years (N=34, 17%). Epithelial cell abnormality was more common in <45 years (N=10), ≥45 years (N=7). The age distribution of lesions by using coefficient of correlation is highly significant with  $p < 0.000068$  (Table 2). Majority of the cases presented with complaints of white discharge (N=142, 71%) followed by menorrhagia in 25 cases (12.5%) and pain abdomen (N=11, 5.5%) (Table 3). Unsatisfactory smears on LBC are less when compared to conventional smears. The coefficient of correlation is significant with  $p$  value of 0.000422 ( $< 0.05$ ).

The number of cases with diagnosis of ASCUS is reported more in liquid-based cytology (9 cases) when compared to conventional Pap (8 cases) probably due to increased size of nucleus on LBC (Figure 2). The number of cases with diagnosis of HSIL (Figure 3), SCC (Figure 4) is reported more in liquid-based cytology (7 cases) when compared to conventional Pap (4 cases). Coefficient of correlation in liquid-based cytology versus conventional Pap smears for NILM is significant with p value of 0.002414 (<0.05), and epithelial cell abnormality is significant with p value of 0.002414 (<0.05) (Table 4). Out of 17 cases of epithelial cell abnormalities on LBC, received biopsy for 5 cases. Correlation was seen in 4 cases, in one case the smear was unsatisfactory on LBC, but HPE showed moderately differentiated squamous cell carcinoma.



**Figure 1: A) Candida yeast forms on conventional Pap smears (PAP stain, 400X), B) Candida yeast forms on liquid based cytology (PAP stain, 400X).**

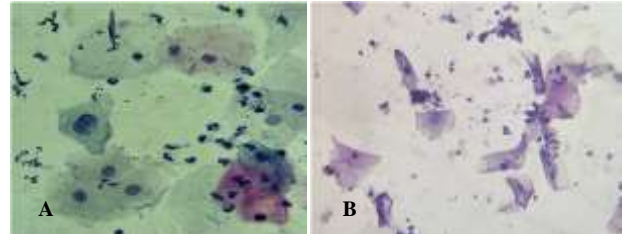
**Table 1: Distribution of cases of cervical lesions on Pap smears by liquid-based cytology, (n=200).**

Lesions	N	% of lesions
NILM- normal	43	21.5
NILM-non specific inflammation	27	13.5
NILM-reactive changes	92	46
NILM-atrophy	4	2
NILM-Trichomonas vaginalis	5	2.5
NILM-Candida	9	4.5
NILM-bacterial vaginosis	1	0.5
ASCUS	8	4
ASC-H	1	0.5
LSIL	1	0.5
HSIL	2	1
HSIL with suspicious of invasion	1	0.5
SCC	4	2
Unsatisfactory smear	2	1

## DISCUSSION

Conventional Pap smear is the most effective method of screening cervical cancer, but its sensitivity varies from 20% to 50%. To overcome the limitations, Liquid-based cytology was introduced in 1990 for better processing of cervical samples. Sangeeta et al analyzed 310 cases of cervical lesions. The most common age group with cervical lesions were between 31-40 years (39%). 47.1% of cases belonged to the lower socioeconomic groups.

The most common complaint was excessive vaginal discharge. On clinical examination, in 52.3% of cases the cervix was normal, and the remaining showing erosion. Epithelial cell abnormality was seen in 2.9% of cases, using conventional Pap smear (CPS) and in 3.87% of cases using liquid-based cytology (LBC) which is not statistically significant. The unsatisfactory smear rate of conventional Pap was 7.1% and on LBC was 1.61%, which is statistically significant.<sup>5</sup>



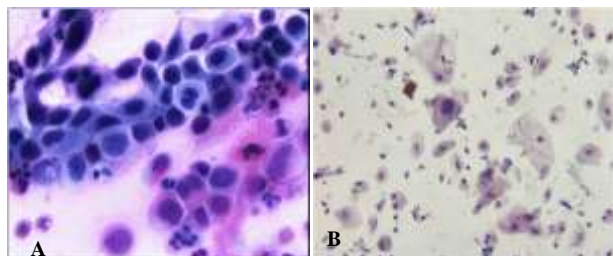
**Figure 2: A) Atypical squamous cells of undetermined significance on CPS (PAP,400X), B) Atypical squamous cells of undetermined significance on LBC (PAP 400X).**

**Table 2: Age distribution of cervical lesions on Pap smears by LBC (n=200).**

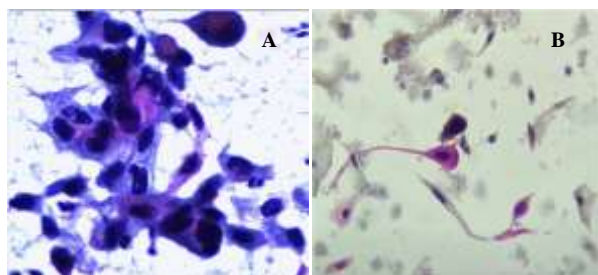
Lesions	N	<45 years	≥45 years
NILM-normal	43	40	3
NILM-non specific inflammation	27	20	7
NILM-reactive changes	92	74	18
NILM-atrophy	4	0	4
NILM-Trichomonas vaginalis	5	5	0
NILM-Candida	9	8	1
NILM-bacterial vaginosis	1	0	1
ASCUS	8	5	3
ASC-H	1	0	1
LSIL	1	1	0
HSIL	2	1	1
HSIL with suspicious of invasion	1	1	0
SCC	4	2	2
Unsatisfactory smear	2	1	1
<b>Total</b>	<b>200</b>	<b>158</b>	<b>42</b>

In the present study epithelial cell abnormality was more common in the age group less than 45 years of age. Majority of the cases presented with complaints of white discharge (71%) followed by menorrhagia in (12.5%) and pain abdomen (5.5%) of cases. In the present study unsatisfactory smears on LBC is less when compared to conventional smears the coefficient of correlation is significant with p value of 0.000422 (<0.05). Epithelial cell abnormality was seen in (8.5%) of

cases. Squamous cell carcinoma in (2%) of cases. In the study by Singh et al, 4.3% were unsatisfactory by conventional method, and 1.7% smears unsatisfactory by LBC technique.<sup>6</sup> In the study by Sangeeta et al the detection rate of epithelial cell abnormality by conventional Pap smear was 2.9% and LBC was 3.87%.<sup>5</sup> Still, in other studies documented by Taylor et al the accuracy of conventional Pap and LBC in detecting epithelial cell abnormality was similar.<sup>7</sup>



**Figure 3: A) HSIL on CPS (PAP stain 400X), B) HSIL on LBC (PAP stain 400X).**



**Figure 4: A) Squamous cell carcinoma on CPS (PAP stain 400X), B) squamous cell carcinoma on LBC (PAP 400X).**

**Table 3: Clinical presentation in cases of cervical lesions (n=200).**

Clinical presentation	N	%
White discharge	142	71
Pain abdomen	11	5.5
Dysmenorrhea	1	0.5
Polymenorrhea	2	1
Oligomenorrhea	1	0.5
DUB	10	5
Menorrhagia	25	12.5
Post menopausal bleeding	3	1.5
Amenorrhea	5	2.5

In the study by Sangeet et al, NILM-normal detected on conventional Pap smear (CPS) was 54.19% and on LBC (64.84%). Inflammatory smears on CPS are 28.7%, and on LBC, 22.9%. NILM-Atrophic smears were 7.1% and on LBC (6.77%). ASCUS were 1.94% on CPS and 2.26% on LBC. ASC-H detected on CPS was nil and on LBC 0.32%. LSIL on CPS (0.32%) and 0.65% on LBC. The comparison between conventional Pap smear and

liquid-based method in various cervical lesions was not significant.<sup>5</sup>

The present study analyzed 200 Pap smears on Liquid-based cytology and compared the results with Conventional Pap smears. The commonest cervical lesions on pap smears by liquid-based cytology are NILM-reactive changes (46%), followed by NILM-Normal (21.5%) and NILM-non specific inflammation (13.5%), NILM-Candida infection is seen in 9 cases (4.5%) and Trichomonas vaginalis infection in 5 cases (2.5%). Coefficient of correlation in liquid-based cytology versus conventional Pap smears for: NILM was significant with a p value of 0.002414 (<0.05). Srivastava et al compared the features of pap smears on Conventional Pap smears with that of LBC. He analyzed 300 cases. In LBC out of 300 cases, 4.7% of cases were unsatisfactory, the main causes being scanty cellularity in 78.6% of cases. NILM was seen in 98.6% of cases, ASCUS in 0.7% cases, LSIL in 0.3% of cases, squamous cell carcinoma in 0.3% of cases, Candida hyphae were seen in 0.3% of cases.<sup>8</sup>

In the present study the number of cases with a diagnosis of ASCUS was reported more in liquid-based cytology (9 cases) when compared to conventional Pap (8 cases) probably due to increased size of nucleus on LBC. Gupta N et al reported that the main cause of unsatisfactory rate in CPS was low cellularity and excess blood.<sup>9</sup> Sieber's et al and Gupta et al found least cellularity as the sole cause for unsatisfactory smears on LBC.<sup>9,10</sup> Bacterial vaginosis was reported in 4% of cases on LBC. Inflammatory cases were more easily diagnosed on CPS.

Sherwani et al analyzed 160 patients with cervical lesions, 48.1% of cases belonged to the 4<sup>th</sup> decade and 31.2% in the third decade. The most common complaint was white discharge in 42.5% of cases, followed by pain abdomen (27.5%) and menstrual irregularity in 23.8% of cases. Unsatisfactory smears on CPS were 5%, and on LBC 3.7%, Candida was seen in 4.3% of cases and Trichomonas vaginalis seen in 4 cases.<sup>11</sup> Sharma et al analyzed 140 cases. 8% were unsatisfactory on CPS and on LBC was 7%.

The reactive changes such as inflammation, atrophy were seen in 9% of cases on CPS and 5% on LBC and are not statistically significant. ASC-H were seen in 3% of cases in CPS and 2% on LBC smears and was statistically significant.<sup>12</sup> Sherwani et al in their study CPS detected organisms only in 3.1% smears and LBC detected organisms in 8.7% of cases.<sup>11,12</sup> Obwegeser et al Sykes et al showed no statistically significant differences between the two techniques in detecting cells ranging from normal to HSIL.<sup>13,14</sup> Obwegeser et al and Davey et al interpretation of ASCUS is more frequent with CPS although no significant difference is seen in LSIL/HSIL detection, this is probably the cells that appear abnormal on CPS and normal in LBC.<sup>13,15</sup>

**Table 4: Correlation between conventional Pap smear and liquid-based cytology in cervical lesions.**

Lesions diagnosed on LBC	N	Lesions diagnosed on conventional Pap smear	Not correlated	P value
<b>NILM-normal</b>	43	38	5	Significant 0.002414 (<0.05)
<b>NILM-non specific inflammation</b>	27	46	19	
<b>NILM-reactive changes</b>	92	67	25	
<b>NILM-atrophy</b>	4	4	0	
<b>NILM-Trichomonas vaginalis</b>	5	3	2	
<b>NILM-Candida</b>	9	11	2	
<b>NILM-bacterial vaginosis</b>	1	1	0	
<b>ASCUS</b>	8	6	2	Significant 0.002414 (<0.05)
<b>ASC-H</b>	1	2	1	
<b>LSIL</b>	1	0	1	
<b>HSIL</b>	2	1	1	
<b>HSIL with suspicious of invasion</b>	1	1	0	
<b>SCC</b>	4	3	1	Significant 0.000422 (<0.05)
<b>Unsatisfactory smears</b>	2	17	15	

In the study by Sharma et al LBC could pick up LSIL and HSIL in 2% of cases but was unable to comment on borderline categories, i.e. ASCUS and ASC-H. LBC was associated with artifactual changes such as rounded squamous cells and pseudo koilocyte change.<sup>12</sup> Sangeetha et al analyzed 50 cases and compared the features between CPS and liquid-based cytology. NILM was seen on CPS was 18% and on LBC 40%. NILM-inflammatory smears 62% on CPS and 20% on LBC. ASCUS was seen in 8% on CPS, LSIL was seen in 8% on CPS and 4% on LBC. HSIL on CPS in 4% of cases and the comparison was highly significant.<sup>16</sup> Fremont et al found sure path method to be significantly superior in detecting high grade squamous intraepithelial lesions and low grade squamous intraepithelial lesions with significant p value, the p value is <0.00001 for each lesion, when compared with conventional slides. Coefficient of correlation in liquid-based cytology versus CPS in detecting epithelial cell abnormality is significant with a p value of 0.002414 (<0.05).<sup>17</sup> Zhu et al analyzed 137 cases of women with atypical pap smears and compared both the techniques, liquid-based cytology and conventional Pap smears. Detection of HSIL was 47% on conventional smears when compared to 66% by liquid based cytology. The proportion of ASCUS was significantly less in liquid based cytology (4.3%) when compared to 8% on conventional pap smears.<sup>18</sup>

Kavatkar et al analyzed 70 cases in which 5.71% cases of Candida, 11.42% cases of bacterial vaginosis, 4.28% cases of Trichomonas vaginalis were identified on liquid-based cytology and 1.42% cases of Candida and 1.42% cases of Trichomonas vaginalis were identified on conventional pap smears. The average primary screening time has reduced from 8 minutes to 4 minutes.<sup>19</sup>

### Limitations

The study was done in a limited number of subjects, because of high cost of laboratory tests. The results may vary if done in a large number of subjects.

### CONCLUSION

Liquid-based cytology has advantages of fewer unsatisfactory smears, less obscuring by blood, mucus material and inflammatory cells. The proportion of ASCUS detected on LBC was more than conventional Pap smear. The total epithelial cell abnormalities detected on conventional Pap smear were 6.5% and on LBC 8.5%. Hence LBC is better for the screening of premalignant and malignant lesions of cervix even though it is costly.

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