

Conventional PAP vs Liquid Based Cytology: A Statistical Analysis at a Tertiary Care Centre

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ABSTRACT

Background: Cervical cancer is the second most common cancer among women in India. Premalignant lesions can be detected through Papanicolaou (Pap) smear screening, reducing morbidity and mortality. Conventional Pap smear (cPAP) was introduced by Dr. Papanicolaou in 1928, while Liquid-Based Cytology (LBC) was introduced as an FDA-approved alternative in 1996. The present study compares LBC and cPAP for cervical neoplasia screening based on adequacy, morphology, and economic feasibility at a tertiary care centre in Central India.

Methods: All female patients, attending the Gynaecologic OPD, who were advised Pap smear screening. About 254 females, who were willing to participate were included in the study. Cervical smears were collected from all patients, using both methods and compared for adequacy, morphology and economic feasibility.

Results: The study included patients aged 23–75 years. Unsatisfactory smears were more frequent with cPAP (9.44%) than LBC (1.18%). LBC detected five additional epithelial abnormalities and showed higher sensitivity and 100% NPV, whereas cPAP had slightly higher specificity (80%) and better visualization of Trichomonas and Candida. Overall, LBC was a more effective screening method.

Conclusion: We conclude that LBC has many advantages over cPAP, however the much higher cost is not justified in our economically challenged patients. It should be used in occasional cases where patient is able to bear the cost, and where Human Papilloma viral DNA testing is indicated.

Key-words: Cervical cancer, HPV, PAP smear, Screening tool, Liquid-Based Cytology (LBC)

INTRODUCTION

Cervical cancer is the second most common cancer in women in India. So detection of its premalignant lesions is of utmost importance, which can be easily done by screening methods. ^[1] Invasive cervical cancer is the end result of a long pathological process that begins with precursor lesion called squamous intraepithelial lesions. ^[2,3]

These neoplastic alterations are causally related to Human Papilloma Virus (HPV) infection, in many of the cases. ^[4,5] Early changes in the cervix in the form of CIN can be detected years before invasive carcinoma develops and this is the basis of effectiveness of cytological screening. Morbidity and mortality from cervical cancers have been shown to decrease substantially by the proper implementation of screening methods. The mainstay of cervical cancer screening has been PAP smear. The main objective of cervical screening is to decrease worldwide incidence and mortality of cervical cancer, by detecting and treating precancerous lesions.

The screening of cervical cytology smears was introduced in 1928 by Dr. George N. Papanicolaou when he reported the observation of dysplastic/malignant cells in women

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with cervical cancer by sampling vaginal smears. Subsequently Papanicolaou and Dr. Herbert Traut identified cells of both invasive and pre invasive cervical neoplastic lesions by cervical cytology [6].

This test is now known as the (cPAP smear or PAP Test For cPAP, cell samples are taken from the cervix by using Ayre's spatula and smeared onto slides, fixed and stained with PAP stain. Specificity of this test is 60%-70%. But the sensitivity, ranges from 50%-75%. Several limitations of conventional pap test are identified such as, inadequate transfer of cells to slide, non-uniform distribution of abnormal cells, presence of obscuring inflammation, blood and overlapping of epithelial cells, and air-drying artefact showing cellular and nuclear enlargement with eosinophilic staining of the nuclei. Liquid based thin layer technology was introduced as a FDA approved alternative method to conventional PAP in 1996 to address these limitations. The first generation automated LBC involves rinsing the sampling device into a vial of fixative to form a suspension of cells from which a monolayer of cells on a slide is prepared. These slides can be read more quickly than cPAP smears and the residual sample can be used for HPV DNA testing. There are two methods of FDA approved LBC technologies- ThinPrep and SurePath. [7-9]

The present study was undertaken to compare cPAP and LBC as screening tools for cervical neoplasia at a tertiary care centre in Central India. The study focused on comparing both methods with respect to smear adequacy and cytomorphological features, while also evaluating their economic feasibility and practical applicability as routine cervical cancer screening methods.

MATERIALS AND METHODS

Study Design and Participants- Observational, cross-sectional comparative, prospective, single-centre study. Patients presenting in the Gynaecology OPD, more than 25 years of age, who were advised a Pap test and were willing to participate in the study were included. Patients below 25 years were included if they were symptomatic. Asymptomatic females presenting in Gynaecology Health Camps in a community setting, willing for cervical cancer screening, were also included in the study.

Inclusion criteria- A comprehensive history including menstrual as well as obstetric history was recorded.

Special mention was made if patients presented with postmenopausal bleeding, postcoital bleeding, early sexual activity before 18 years of age, multiple sexual partners, multiparity, white discharge per vaginum, or an unhealthy cervix on speculum examination. General examination findings, diagnostic gynaecological findings on per-speculum and per-vaginal examination, radiological findings, where available, and probable diagnosis were recorded for all subjects.

Exclusion Criteria- Patients having abnormal vaginal bleeding with a known uterine or hormonal cause and known cases of invasive carcinoma of the cervix were excluded from the study.

Data Collection and Sample Processing- The research case proforma included study case number, name, age, gender, MRD number, chief complaints, past history, menstrual history, obstetric history, personal history, examination findings including per-speculum and per-vaginal examination, cPAP findings as per the Bethesda System of Reporting Cervical Cytology, LBC findings as per the Bethesda System of Reporting Cervical Cytology, and histopathology findings, where available.

For conventional PAP, cyto-brush, Ayre's spatula, glass slide, fixative, distilled water, and PAP stain were used. For SurePath Liquid-Based Cytology, Rover's Cervix Brush, SurePath™ preservative vials/solutions, SurePath™ direct-to-slide kit with settling chambers, SurePath precoated slides, density reagent, cytology stain kit for gynaecological haematoxylin stain, bottle alcohol blend rinse, vortex mixer, and centrifuge were used.

Paired samples were collected from all enrolled subjects. They were compared in respect of adequacy, cellularity, background, distribution of material, cell overlapping, nuclear changes, cytoplasmic changes and interpretation of results. For reporting, the Bethesda System 2021 was used in both the methods. In cases where follow-up histology was available, either cervical biopsy (colposcopic or direct) or hysterectomy, it was used as a gold standard for calculating the sensitivity of the two methods. Any grade of epithelial abnormality obtained on histology was considered as true positive, even if it did not match the grade reported on cytology. The patient data were collected using a patient proforma as

mentioned above, tabulated, and results were analysed by using appropriate statistical methods.

Statistical Analysis- Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for both methods using appropriate formulae. McNemar's chi-square test was applied to compare the paired categorical diagnostic outcomes of cPAP and LBC against histopathology in the same study population. This test was considered appropriate for evaluating differences between the two paired screening methods. Statistical analysis was performed using SPSS version 24.0.

Ethical Considerations- Institutional Ethics Committee approval was obtained before commencement of the study. Written informed consent was obtained from all participants, with the consent form provided in the local language (Marathi). All participant-related data were maintained in strict confidentiality, and study-specific codes were used for identification of cases to ensure privacy and confidentiality.

RESULTS

A total of 254 subjects, including suspected cases of cervical lesions as well as asymptomatic women, were included in the study. The age of the study subjects ranged from 23 to 75 years, with the maximum number of subjects belonging to the 25–60-year age group. The demographic distribution of the study population is presented in Table 1.

Table 1: Age distribution of study cases.

Age (years)	No of subjects	% age
20-30	50	19.68
31-40	66	26
41 – 50	72	28.34
51 – 60	56	22
61 – 70	08	3.20
71 - 80	02	0.78

The commonest presenting complaint was white discharge per vaginum, reported in 128 (50.39%) subjects, followed by mucopurulent discharge in 52 (20.48%) subjects and irregular menstruation in 38 (14.96%) subjects. The commonest finding on per-vaginal

and per-speculum examination was mucoid and mucopurulent discharge. Other examination findings included cervical erosion, bleeding on touch, genital wart, cervical polyp, and fungating growth, while no abnormality was detected in 52 subjects. The presenting symptoms and examination findings are summarized in Table 2.

Table 2: Symptomatic and Per Vaginal/Per Speculum Examination Findings at Presentation

Findings	No.	Percentage (%)
Symptoms at presentation		
White discharge	128	50.39
Irregular menstruation	38	14.96
Postcoital bleeding	12	4.73
Postmenopausal bleeding	5	1.96
Mucopurulent discharge	52	20.48
Itching at the vulva	9	3.54
Pain abdomen	10	6.50
Per Vaginal and Per Speculum Examination Findings		
Mucoid discharge	98	38.58
Mucopurulent discharge	42	16.54
Cervical erosion	18	7.10
Cervical polyp	7	2.76
Bleeding on touch	24	9.44
Genital wart	10	3.93
Fungating growth	3	1.18
No abnormality detected	52	20.47

Among the cPAP smears, 24 (9.44%) were unsatisfactory for evaluation. The main causes were obscuring of cellular morphology by inflammatory infiltrate or blood, followed by paucity of squamous cells. In comparison, only 3 (1.18%) LBC smears were unsatisfactory, predominantly because of paucity of cellularity. The cytological diagnoses obtained by both methods are presented in Table 3. The representative cytological appearance of obscured morphology in cPAP is shown in Fig. 1.

Table 3: Cytologic diagnoses by both methods.

Cytology finding	Conventional PAP	Liquid based cytology
Unsatisfactory for evaluation	24 (9.44%)	03 (1.18%)
Obscured by inflammatory infiltrate	11 (4.33%)	-
Obscured by blood	08 (3.13%)	-
Paucity of squamous cells	05 (1.97%)	03 (1.18%)
Transformation zone element seen	125 (49.21%)	248 (97.63%)
Negative for Intraepithelial Lesion or Malignancy		
Normal	34 (13.39%)	45 (17.72%)
Atrophic	56 (22.04%)	50 (19.68%)
Benign cellular changes of inflammation	74 (29.13%)	87 (34.25%)
Candida	08 (3.14%)	04 (1.58%)
Trichomonas	03 (1.18%)	02 (0.79%)
Herpes simplex virus	01 (0.39%)	-
Changes of repair and regeneration	13 (5.11%)	17 (6.69%)
Total	187 (73.62%)	208 (81.88%)
Epithelial cell abnormalities		
ASCUS*	09 (3.54%)	09 (3.54%)
ASC-H†	07 (2.75%)	05 (1.97%)
LSIL‡	13 (5.11%)	15 (5.90%)
HSIL§	07 (2.75%)	10 (3.94%)
SCC	03 (1.18%)	04 (1.58%)
Atypical glandular cells	02 (0.79%)	03 (1.18%)
Adenocarcinoma	-	-
Total	41	46

ASCUS* – Atypical squamous cells of undetermined significance. ASC-H†- Atypical squamous cells, cannot rule out HSIL, LSIL‡ – Low grade squamous intraepithelial lesion, HSIL§ – High grade squamous intraepithelial lesion, SCC || – Squamous cell carcinoma.

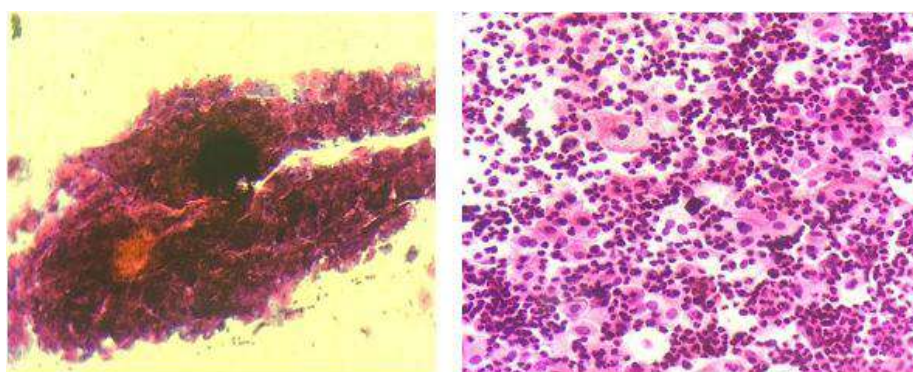


Fig. 1: cPAP (×100 and ×400): Marked overlapping of cells and dense inflammatory infiltrate obscuring cellular morphology

Transformation-zone elements, including endocervical cell groups or metaplastic squamous cells, were identified in 125 (49.21%) cPAP smears, compared with 248 (97.63%) LBC smears, demonstrating a higher proportion of transformation-zone representation in LBC.

Microorganisms identified on cytological examination included Candida, Trichomonas, and herpes simplex virus. Candida and Trichomonas were better visualized in cPAP smears. The characteristic cytological appearances of Candida and herpes simplex virus are demonstrated in Fig. 2 and Fig. 3, respectively.

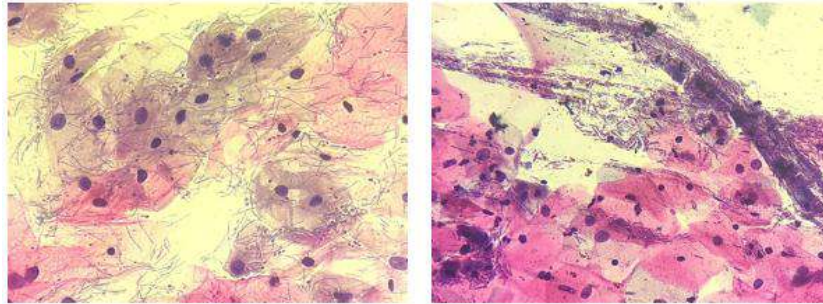


Fig. 2: cPAP (×400): Lactobacilli and budding yeast forms of *Candida* with pseudohyphae

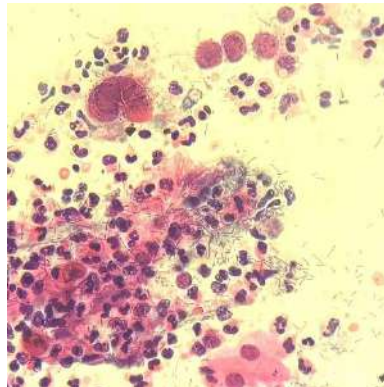


Fig 3: cPAP X400. Herpes simplex virus. Multinucleation, nucleomegaly, moulding and ground glass chromatin

LBC detected five more cases of epithelial cell abnormalities than cPAP. The epithelial abnormalities identified included ASC-US, ASC-H, LSIL, HSIL, SCC, and atypical glandular cells. The corresponding cytological findings are illustrated in Fig. 4 to Fig. 6. The additional

cases of LSIL, HSIL, and SCC detected by LBC occurred in smears in which the corresponding cPAP smears were inadequate for interpretation because the cellular morphology was obscured by blood or inflammation.

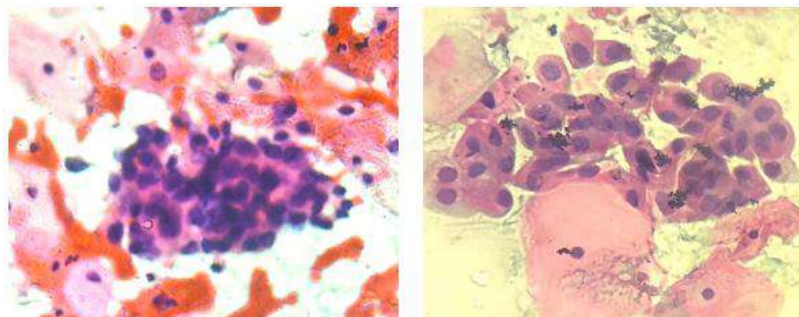


Fig. 4: cPAP and LBC (×400): Atypical glandular cells of undetermined significance showing cellular crowding, nucleomegaly, and hyperchromasia in metaplastic cells.

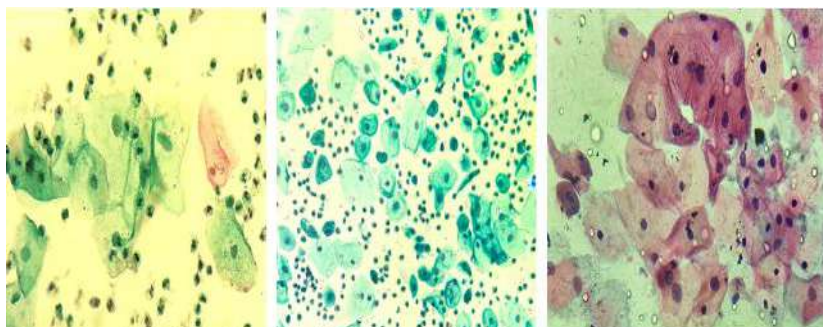


Fig. 5: cPAP and LBC (×400): ASC-US and LSIL showing nucleomegaly, nuclear membrane irregularity, irregular chromatin, and koilocytosis.

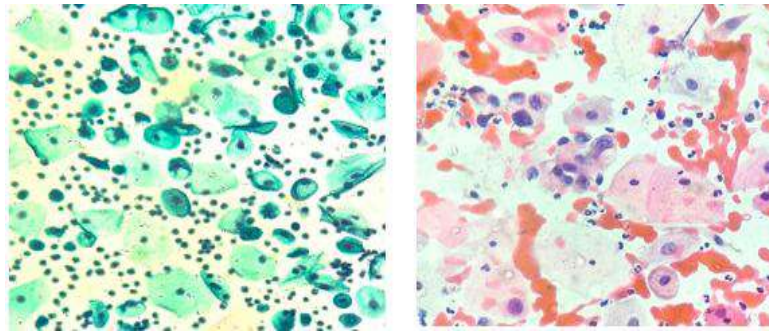


Fig. 6: LBC and cPAP (×400): HSIL showing koilocytosis, marked nucleomegaly, hyperchromasia, and irregular nuclear membranes in basal-like cells

Histopathology specimens were available in 22 cases. Using histopathology as the gold standard, the cPAP group showed one false-negative and two false-positive cases. The false-negative case was reported as NILM on cPAP but was diagnosed as CIN 1 on histopathology. One case reported as ASC-H and one case reported as HSIL on cPAP were negative on histopathological examination. The cytohistological correlation and diagnostic performance of cPAP and LBC are presented in Table 4.

McNemar's chi-square test was applied to statistically compare the diagnostic effectiveness of cPAP and LBC, as both screening methods were evaluated against histopathology in the same study population. Both methods demonstrated high diagnostic accuracy. LBC showed higher sensitivity and NPV, both reaching 100%, whereas cPAP demonstrated slightly higher specificity (80%). The diagnostic performance of both methods is included in Table 4.

Table 4: Cytohistological Correlation and Diagnostic Accuracy of Conventional Pap Smear and Liquid-Based Cytology

Bethesda Category	No.	Histology Received	Normal/ Inflammatory	CIN 1	CIN 2/3/ CIS	Carcinoma
Conventional Pap						
NILM	187	06	06 (TN)	-	01 (FN)	-
ASC-US	09	02	-	01 (TP)	01 (TP)	-
ASC-H	07	01	01 (FP)	-	-	-
LSIL	13	04	01 (FP)	03 (TP)	-	-
HSIL	07	03	-	-	02 (TP)	01 (TP)
SCC	03	03	-	-	-	03 (TP)
Unsatisfactory	24	02	02 (TN)	-	-	-
Liquid-Based Cytology						
NILM	208	06	06 (TN)	-	-	-
ASC-US	07	02	-	01 (TP)	01 (TP)	-
ASC-H	05	02	01 (FP)	-	01 (TP)	-
LSIL	15	06	01 (FP)	04 (TP)	01 (TP)	-
HSIL	09	03	-	-	02 (TP)	01 (TP)
SCC	04	03	-	-	-	03 (TP)
Unsatisfactory	03	0	-	-	-	-

TP = True Positive; FP = False Positive; TN = True Negative; FN = False Negative.

Conventional Pap smear: Sensitivity = 91.67%; Specificity = 80%; PPV = 84.62%; NPV = 88.89%.

Liquid-Based Cytology: Sensitivity = 100%; Specificity = 75%; PPV = 87.50%; NPV = 100%.

DISCUSSION

Carcinoma cervix, the second most cancer among women is an entity that has been described as “preventable but not prevented”. It develops through

sequential stages of dysplasia, from low grade to high grade, carcinoma in situ to invasive cancer, over a variably prolonged interval. ^[1-3] HPV infection is causally related to this cancer and this is the basis of the HPV



DNA test used in cancer cervix screening^[4,5]. Also it is an easily accessible organ that can be screened with minimal invasion. The cPAP smear test has been the backbone of cervical cancer screening programs, which has yielded rich dividends in developing countries, where it has been implemented well. It has been responsible in down staging carcinoma cervix to a very great extent^[7-9]. The cPAP suffers from some drawbacks, in the form of inadequate sampling, incomplete transfer of cells onto the slide, obscuring of morphology by blood and inflammatory infiltrate or overlapping of cells due to improper spreading, drying artefacts, etcetera, that prevent an adequate interpretation of the smear.

LBC has been developed to take care of all these drawbacks of cPAP. It involves transferring the sampled material into a vial of special fluid, which ensures complete transfer of cells and prevents drying, at the same time clearing blood and inflammatory infiltrate. The fluid is then mechanically transferred onto the slide on a small area, with uniform spreading. This ensures fast screening of the whole material saving time and effort^[10]. Additionally, the sample can be stored at room temperature for one month and in the refrigerator for six months, and used for ancillary tests like Human Papilloma Virus DNA testing.

LBC, however is several times more expensive and requires a special setup. This study is, therefore, a comparative analysis of cPAP and LBC as screening methods for cervical cancer, to recommend whether cPAP should or should not be replaced by LBC in our conditions.

We found no statistically significant differences in the sensitivity, specificity and PPV of the two methods, with only a slightly greater NPV in case of LBC. This was in an accordance with the findings of Agarwal *et al.*^[11], Sharma *et al.*^[12], and Pankaj *et al.*^[13]. We found, similar to these authors, that there was significant decrease in the rate of unsatisfactory smears with LBC in comparison to cPAP and increased speed of reporting, but this is not enough to justify the marked increase in cost incurred for LBC.

Anushree *et al.*^[14], on the other hand reported in a study from western Sweden, that LBC yielded significantly more high-grade lesions compared with cPAP smears. Patel *et al.*^[15] reported that LBC would make a significant difference in the morbidity and mortality of carcinoma cervix in settings where very high numbers are being

screened, with the provision of doing human papillomavirus-based testing on the remaining sample.

CONCLUSIONS

The present study demonstrates that both cPAP smear and liquid-based cytology (LBC) are effective methods for cervical cytological screening. However, LBC showed a clear advantage in terms of sample adequacy, with a markedly lower proportion of unsatisfactory smears compared with cPAP. LBC also provided better representation of transformation-zone elements and detected five additional cases of epithelial cell abnormalities, including additional cases of LSIL, HSIL, and SCC that were difficult to interpret on corresponding cPAP smears because of obscuring blood or inflammation. Histopathological correlation further demonstrated high diagnostic performance of both methods. LBC showed 100% sensitivity and 100% negative predictive value, while cPAP showed slightly higher specificity. Overall, the findings indicate that LBC may provide improved specimen adequacy and detection of cervical epithelial abnormalities compared with conventional Pap cytology, supporting its use as an effective screening modality for cervical lesions.

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