ISSN- O: 2458 - 868X, ISSN–P: 2458 – 8687
National Library of Medicine - ID: 101731606

International Journal of Medical Science and Innovative Research (IJMSIR)
IJMSIR : A Medical Publication Hub
Available Online at: www.ijmsir.com
Volume – 10, Issue – 4, August – 2025, Page No. : 128 – 139

A Prospective Observational Comparative Study of Liquid-Based Cytology and Pap Smears

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Citation this Article: Dr. Vaibhavi Umesh Dhenge, Dr. Kishorkumar Hol, Dr Aditi Gore, Dr Vanishree Chavhan, "A Prospective Observational Comparative Study of Liquid-Based Cytology and Pap Smears", IJMSIR - August – 2025, Vol – 10, Issue - 4, P. No. 128 – 139.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Liquid-Based Cytology and Pap Smears: Which is the Better Choice?"

Objectives: This study aimed to compare the cytomorphological differences between Liquid-Based Cytology (LBC) and Conventional Pap Smear (CPS). Additionally, it sought to evaluate the relative advantages and limitations of both techniques.

Materials: A total of 300 cases were analyzed. Conventional Pap Smear samples were collected using a cytobrush, while the cervical brush head was suspended in an LBC vial and processed using the Sure Path system.

Results: The study involved participants aged 31 to 80 years, with a mean age of 54.1 years. Among the 300 cases, 280 satisfactory smears were obtained using CPS, compared to 292 using LBC. Inflammatory smears were detected in 204 cases (68%) with CPS and 219 cases (73 %) with LBC. CPS identified 9 cases of Low-Grade Squamous Intraepithelial Lesion (LSIL) and 3 cases of High-Grade Squamous Intraepithelial Lesion (HSIL), while LBC identified 9 cases of LSIL, 6 cases of HSIL, and 2 cases of squamous cell carcinoma. Histopathological correlation was conducted in 20 cases, where LBC demonstrated 100% sensitivity and specificity.

Conclusion: LBC outperformed CPS in terms of adequacy, background clarity, infection detection, and sensitivity and specificity for identifying LSIL and HSIL. These findings suggest that LBC is a superior method for cervical cytology.

Keywords: Cervical Cancer, Cervical Brush Conventional Pap Smear Sensitivity

Introduction

Cervical cancer is a growing global health concern and a significant cause of mortality among women. World Cancer Statistics report that over 80% of cervical cancer cases occur in developing and low-resource countries, primarily due to limited awareness and challenges in implementing cytology-based screening programs [1]. In India, cervical cancer is the second most common malignancy among women aged 15–44 years [2].

For decades, the conventional Pap smear has been a key tool in cervical cancer screening and has significantly contributed to the early detection and prevention of cervical malignancies [3]. However, this method has notable limitations. Suboptimal smears often result from insufficient squamous cell sampling, obscuring factors such as blood and mucin, dense inflammation, or overlapping epithelial cells on thick smears. These issues lower the sensitivity of the technique and contribute to higher false-negative rates [4,5].

To overcome these limitations, liquid-based cytology (LBC) was developed as a thin-layer technology. This method addresses the shortcomings of the conventional Pap smear by improving smear adequacy and reducing artifacts caused by blood and mucus [6–8].

Methodology

Study Design and Setting

A prospective, observational, comparative study was conducted in the Department of Obstetrics and Gynaecology at the Smt. Kashibai Navale Medical College and General Hospital, a tertiary care center. The study was carried out over a period of five months, from February 2025 to June 2025, and included 300 women attending the gynecology outpatient department (OPD) [9].

Study Population

Eligible participants were women presenting with gynecological complaints such as vaginal bleeding, white discharge, irregular menstruation, lower abdominal pain, and postcoital bleeding [10].

Inclusion Criteria

The study included women aged 31 years and older who presented with symptoms indicative of cervical lesions or an unhealthy cervix. These symptoms included:

- Vaginal bleeding
- Cervical ulceration
- Abnormal white discharge
- Irregular menstrual cycles
- Lower abdominal pain
- Postcoital bleeding

Exclusion Criteria

The following groups were excluded from the study:

- Women who had undergone a total hysterectomy
- Pregnant women

Sample Collection

After obtaining informed written consent, cervical samples were collected using a cytobrush. The brush was rotated 360° and smeared on a glass slide, and a cervical brush sample was taken for LBC [11].

1. **Conventional Pap Smear (CPS):** The sample was smeared onto a glass slide and immediately fixed in alcohol for cytological analysis [12].
2. **Liquid-Based Cytology (LBC):** The sample from the cervical brush was suspended in a vial containing 14 mL of EziPREP preservative fluid, specifically prepared for the SurePath method. The vial was labeled, capped, and sent to the laboratory for processing [13,14].

Laboratory Procedure (LBC)

- **Centrifugation:** Approximately 4 mL of separator solution was layered with 7 mL of preservative fluid in a centrifuge tube. Samples were centrifuged at 1500–2000 rpm for 5 minutes [15].

- **Supernatant Removal:** The supernatant was discarded, and the remaining sample was transferred to the EziPREP Nanocyt Neo machine [16].
- **Slide Preparation:** A micropipette was used to place 50–75 µL of the sample on a slide, creating a monolayer of cells. The EziPREP Nanocyt Neo, an advanced cytology centrifuge with filterless technology, produced dual smears (16 mm diameter each) on the slide, optimizing cellular morphology for analysis [16].

Staining

Both conventional Pap smear slides and LBC slides were stained using the rapid Pap method (Ultra Pap Kit) [17].

This methodology ensured high-quality sample preparation and enabled a comparative cytological evaluation between conventional Pap smears and LBC slides.

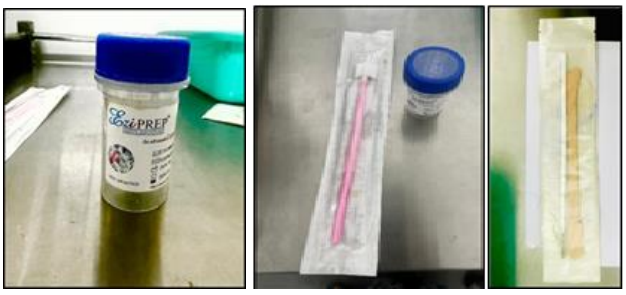


Figure 1: wooden spatula cytobrush for CPS and sample brush and vial containng Eziprep for LBC

Chart 1: Age wise distribution of participants

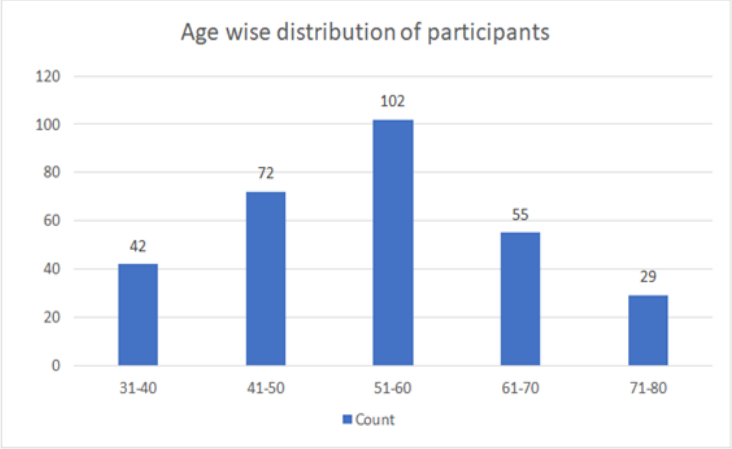


Table 1: AGE wise distribution

Category	Subcategory	Count	Percentage (%)
Age	31–40	42	14.0%
	41–50	72	24.0%
	51–60	102	34.0%
	61–70	55	18.3%
	71–80	29	9.7%
Total		300	100%

Chart 2: Complaints of participants.

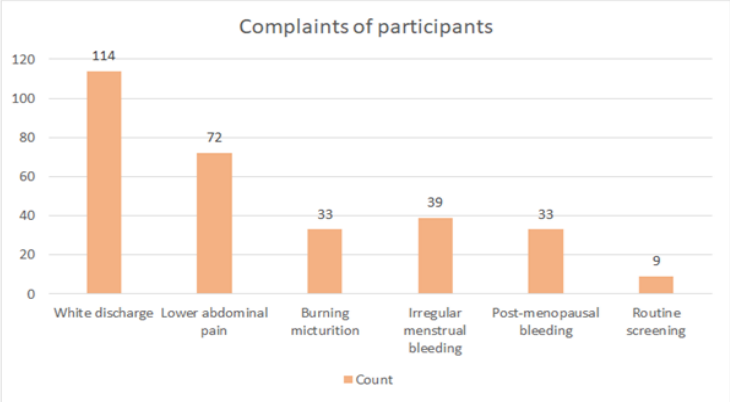


Table 2: distribution according to the complaints

Category	Subcategory	Count	Percentage (%)
Complaints	White discharge	114	38
	Lower abdominal pain	72	24
	Burning micturition	33	11
	Irregular menstrual bleeding	39	13
	Post-menopausal bleeding	33	11
	Routine screening	9	3

Chart 3: Pre Speculum Findings

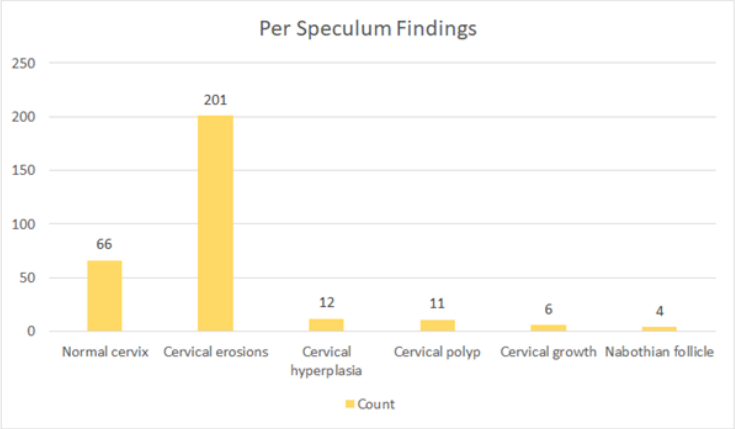


Table 3: distribution according the per speculum findings

Category	Subcategory	Count	Percentage (%)
Per Speculum Finding	Normal cervix	66	22
	Cervical erosions	201	67
	Cervical hyperplasia	12	4
	Cervical polyp	11	3.8
	Cervical growth	6	2
	Nabothian follicle	4	1.2

Statistical Analysis

Data analysis was performed using SPSS software version 21.0. Categorical variables were analyzed using the Chi-square test. A p-value of less than 0.05 was considered statistically significant.

Study Overview

- Total Patients: 300
- Age Range31-80 years (Mean: 54.1 years)
- Age Distribution:

The majority belongs to the 51–60 years age group (34%)

Parity Distribution

- **Cases:**
  - 150 cases (50%) had 2 children.
  - 100 cases (33.3%) had 3 children.
- **Clinical Presentation**
  - **Common Complaints:**
    a) White discharge: 114 cases (38%)
    b) Lower abdominal pain: 72 cases (24%)
- **Per Speculum Findings**
  - **Findings:**
    a) Cervical erosions: 201 cases (67%)
    b) Cervical hypertrophy: 12 cases (4%)
- In the evaluation of specimen adequacy, the conventional Pap smear (CPS) had 280 satisfactory cases (93.33%) and 20 unsatisfactory cases (6.67%). In contrast, liquid- based cytology (LBC) demonstrated 292 satisfactory cases (97.33%) and 8 unsatisfactory cases (2.67%). Despite the slight differences in adequacy rates between the two methods, the results were statistically significant, as indicated by a p-value of 0.033.
- For the cytological findings of CPS, 51 cases (17.0%) were classified as normal, while 204 cases (68.0%) were classified as inflammatory.

Chart 4: Specimen adequacy in LBC VS CPS

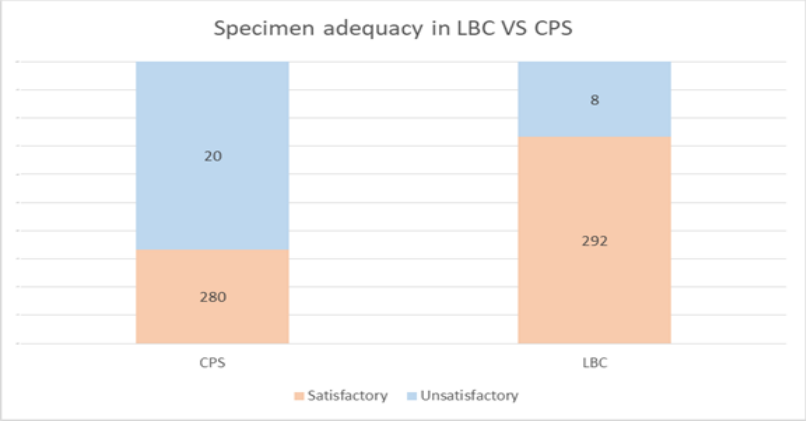


Table 4: Distribution of CPS Findings (Normal vs. Inflammatory Smears)

Cytological Findings	Conventional Pap Smear (CPS)
Normal	51 (17%)
Inflammatory	204 (68%)

Table 5: Comparison of Specimen Adequacy between Conventional Pap Smear (CPS) and Liquid-Based Cytology (LBC)

Specimen Adequacy	Conventional Pap Smear (CPS)	Liquid-Based Cytology (LBC)
Satisfactory	280 (95.4%)	292 (97.4%)
Unsatisfactory	20 (6.67%)	8 (2.67%)
Total	300 (100%)	300 (100%)

- In the study, 48 cases (16.0%) were classified as normal and 221 cases (73.67%) as inflammatory in liquid-based cytology (LBC).
- For negative intraepithelial lesions, bacterial vaginosis was observed in an equal number of cases in both CPS and LBC. However, Candida and Trichomonas vaginalis were identified more frequently using LBC.

When comparing epithelial cell abnormalities:

- **ASCUS (Atypical Squamous Cells of Undetermined Significance):** 5 cases were reported using CPS, while 6 cases were reported using LBC.
- **LSIL (Low-Grade Squamous Intraepithelial Lesion):** 10 cases were identified via CPS, while 9 cases were identified via LBC.
- **Squamous Cell Carcinoma (SCC):** 1 case was detected with CPS, while 2 cases were detected with LBC.

Histopathological Correlation

Histopathological evaluation was performed on 20 patients:

Out of 6 cases reported as Atypical Glandular Cells of Undetermined Significance (AGUS) on cytology, all underwent colposcopy with endocervical curettage (ECC) and endometrial sampling. Histopathological correlation revealed:

- 2 cases (33.3%) showed mild dysplasia (CIN I).
- 1 case (16.7%) revealed moderate dysplasia (CIN II), which was correctly diagnosed by Liquid-Based Cytology (LBC).
- 3 cases (50%) were negative for cervical intraepithelial neoplasia (CIN) and glandular neoplasia. These patients were advised routine screening and follow-up.

1. LSIL Findings:

Out of 9 patients with Low-Grade Squamous Intraepithelial Lesion (LSIL) on cytology:

7 cases (77.8%) were confirmed histopathologically as mild to moderate dysplasia (CIN I–II).

1 case (11.1%) revealed severe dysplasia (CIN III).

1 case (11.1%) was diagnosed as squamous cell carcinoma (SCC), which was correctly detected by Liquid-Based Cytology (LBC).

2. HSIL Findings:

Out of 3 patients diagnosed with High-Grade Squamous Intraepithelial Lesion (HSIL) by Conventional Pap Smear (CPS), histopathology revealed:

2 cases (66.7%) as *severe dysplasia (CIN III).*

1 case (33.3%) as adenocarcinoma, which was correctly detected by Liquid-Based Cytology (LBC).

3. SCC Findings:

Among 4 patients screened as *Squamous Cell Carcinoma (SCC)* by Liquid-Based Cytology (LBC), histopathology showed:

- 3 cases (75%) confirmed as *SCC*.
- 1 case (25%) revealed *adenocarcinoma*.

When correlated with Conventional Pap Smear (CPS):

- 2 cases were correctly screened as *SCC*.
- 1 case was misinterpreted as *LSIL*.
- 1 case was misinterpreted as *HSIL*.

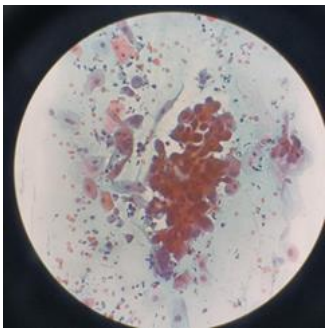


Figure 2: Adenocarcinoma

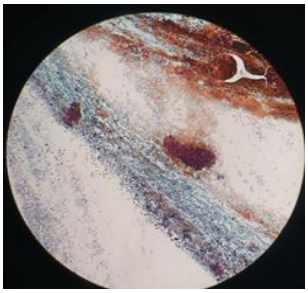


Figure 3: HSIL

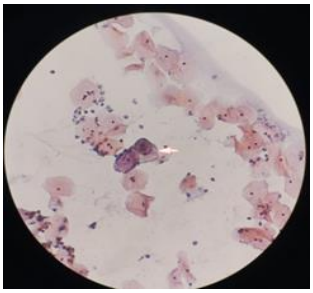


Figure 4: Clue cells in bacterial vaginosis

Table 6: CPS vs. LBC interpretations, and correlation with histopathology findings

Cytology Category	No. of Cases	CPS Interpretation	LBC Interpretation	Histopathology Findings	Remarks
AGUS (Atypical Glandular Cells of Undetermined Significance)	6	Reported as AGUS	Reported as AGUS	2 – CIN I (mild dysplasia) 1 – CIN II (moderate dysplasia, correctly diagnosed by LBC) 3 – Negative for CIN/glandular neoplasia	Routine screening advised for negative cases
LSIL (Low-Grade Squamous Intraepithelial Lesion)	9	Diagnosed as LSIL	Diagnosed as LSIL	7 – CIN I/II (mild to moderate dysplasia) 1 – CIN III (severe dysplasia) 1 – SCC (correctly identified by LBC)	High concordance with LBC
HSIL (High-Grade Squamous Intraepithelial Lesion)	3	Diagnosed as HSIL	Diagnosed as HSIL	2 – CIN III (severe dysplasia) 1 – Adenocarcinoma (correctly identified by LBC)	LBC superior in glandular lesion detection
SCC (Squamous Cell Carcinoma)	4	2 – Correctly identified as SCC 1– Misinterpreted as LSIL 1 – Misinterpreted as HSIL	All 4 diagnosed as SCC	3 – SCC 1 – Adenocarcinoma	LBC showed higher diagnostic accuracy

Diagnostic Performance of LBC

In predicting squamous cell carcinoma, LBC demonstrated:

Table 7: Comparison of Diagnostic Accuracy between CPS and LBC

Test	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
CPS	84.21	98.93	84.21	98.93
LBC	100.00	99.29	90.48	100.00

Discussion

The present study compared the diagnostic performance of Conventional Pap Smear (CPS) and Liquid-Based Cytology (LBC) with histopathology as the gold standard in cervical cancer screening. Out of 300 women screened, epithelial cell abnormalities were detected in both CPS and LBC, with a higher detection rate for significant lesions by LBC.

In our analysis, LBC demonstrated superior sensitivity (100%) compared to CPS (84.2%), indicating that LBC was more effective in correctly identifying true positive cases of cervical intraepithelial neoplasia and carcinoma. The slightly lower sensitivity of CPS is attributable to its tendency to miss higher-grade lesions, as seen in cases misclassified as LSIL or HSIL instead of SCC or adenocarcinoma.

Specificity was high for both methods (98.9% for CPS vs. 99.3% for LBC), reflecting their reliability in ruling out disease when cytology results were negative. However, LBC had a marginal advantage due to fewer false positives, especially in cases initially reported as AGUS on CPS.

Positive predictive value (PPV) was higher in LBC (90.5%) compared to CPS (84.2%), showing that a positive diagnosis by LBC is more likely to reflect true disease on histopathology. Importantly, negative predictive value (NPV) reached 100% with LBC, eliminating the risk of missed lesions in negative cases, while CPS had an NPV of 98.9%.

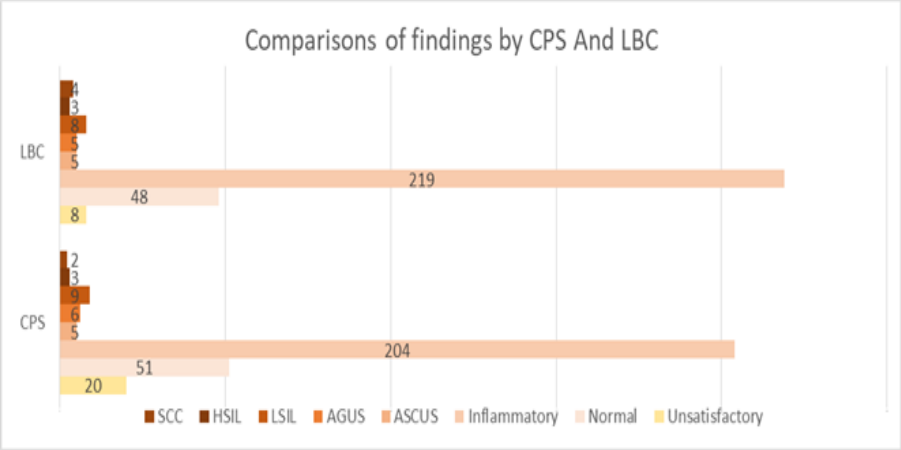
Our findings are consistent with previous studies, which have reported that LBC improves sample adequacy, reduces unsatisfactory smears, and enhances detection of both squamous and glandular lesions [12,17–19]. In particular, the ability of LBC to accurately identify adenocarcinoma, which was misclassified by CPS, highlights its diagnostic superiority [20,21].

From a public health perspective, the higher sensitivity and NPV of LBC make it a more reliable screening tool for cervical cancer prevention programs. However, CPS remains widely used in low-resource settings due to its lower cost and wider availability [3,16].

Table 8: Comparative Cytological Findings in CPS and LBC

CPS				LBC		
	Sample	Percentage			Sample	Percentage
Unsatisfactory	20	6.67%		Unsatisfactory	8	2.67%
Normal	51	17.00%		Normal	48	16.00%
Inflammatory	204	68.00%		Inflammatory	219	73.00%
ASCUS	5	1.67%		ASCUS	5	1.67%
AGUS	6	2.00%		AGUS	5	1.67%
LSIL	9	3.00%		LSIL	8	2.67%
HSIL	3	1.00%		HSIL	3	1.00%
SCC	2	0.67%		SCC	4	1.33%
Total	300	100.00%		Total	300	100.00%

Chart 5: Comparisons of findings by CPS and LBC



The p-value for the comparison of specimen adequacy between Conventional Pap Smear (CPS) and Liquid-Based Cytology (LBC) is 0.033.

This indicates a statistically significant difference ($p < 0.05$), showing that LBC yields a significantly higher proportion of satisfactory samples compared to CPS.

Table 9: Comparison of Specimen Adequacy in Conventional Pap Smear (CPS) and Liquid-Based Cytology (LBC) Across Various Studies

Author/Study	Year	Total Cases	Satisfactory CPS (%)	Satisfactory LBC (%)
Costa et al. [17]	2015	525	98.29	95.62
Singh et al. [18]	2015	80	95.7	98.3
Stabile et al. [19]	2015	100	98	99
Sharma et al. [5]	2016	140	92	93
Pankaj et al. [20]	2018	310	92.9	98.4
Aboobacker & Shariff [21]	2020	240	92	95
Manjunath & Sheetal [22]	2021	120	93.6	98.8
Present study	2025	300	95.4	97.4

Enhanced Diagnostic Accuracy and Treatment Approaches in Cervical Screening

Our findings are consistent with previous studies, which have reported that LBC improves sample adequacy, reduces unsatisfactory smears, and enhances detection of both squamous and glandular lesions [12,17–19]. In particular, the ability of LBC to accurately identify adenocarcinoma, which was misclassified by CPS, highlights its diagnostic superiority [20,21].

From a public health perspective, the higher sensitivity and NPV of LBC make it a more reliable screening tool for cervical cancer prevention programs. However, CPS remains widely used in low-resource settings due to its lower cost and wider availability [3,16].

Integration of Cytology and HPV Testing

For high-risk cases, the combination of cytology and HPV testing remains a valuable screening strategy. Cytological findings guide the next steps, while

histopathological correlation through tissue sampling provides the definitive diagnosis. For cervical intraepithelial neoplasia (CIN), management varies based on the grade:

- **CIN 1:** Observation and co-testing repeated annually is an acceptable approach.
- **CIN 2 or higher:** Treatment is typically required, utilizing either ablative or excisional techniques.

Treatment Modalities for CIN

Ablation (tissue destruction, no specimen for histology)

- **Indications:** Noninvasive, ectocervical lesions (mainly CIN 1 or selected CIN 2). SCJ and lesion fully visible, endocervical sampling negative for HSIL.
- **Contraindications:** Previous treatment, glandular abnormality, AIS, suspicion of invasive cancer.
- **Techniques:**

➢ **Cryotherapy:** Freezes lesion with nitrous oxide probe. Best for small, ectocervical lesions. Less effective for CIN 3 or HIV-positive women.

➢ **CO_2 Laser Ablation:** Vaporizes tissue to 5–7 mm depth under colposcopy. Useful for large/irregular lesions, condylomas, or vaginal extension.

- **Limitation:** No specimen for histology → occult invasive cancer cannot be ruled out.

Excision (tissue removal, specimen available for histology)

- **Indications:** Higher-risk lesions (CIN 2/3, AIS, ASC-H/HSIL/AGC cytology, non-visible SCJ, endocervical involvement, recurrent/persistent CIN).
- **Advantages:** Provides histology and margin assessment → excludes invasive cancer.
- **Techniques:**

➢ **LEEP / LLETZ:** Most common; outpatient, local anesthesia, customizable loop sizes; may cause margin thermal damage.

➢ **Cold-Knife Conization (CKC):** Preferred when invasive cancer suspected (large CIN 3, AIS, older age). Higher risks: bleeding, cervical incompetence, preterm birth.

➢ **Laser Conization:** Rarely used; precise and less bleeding, but costly and requires expertise.

- **Risks:** Bleeding, infection, cervical stenosis, adverse pregnancy outcomes (preterm birth, cervical insufficiency).

Conclusion

The present study demonstrated that Liquid-Based Cytology (LBC) offers significant advantages over the Conventional Pap Smear (CPS) in cervical cancer screening. LBC showed superior performance in terms of specimen adequacy, background clarity, infection detection, and diagnostic accuracy for epithelial cell abnormalities. While CPS detected squamous lesions effectively, it missed several high-grade lesions and one adenocarcinoma that were correctly identified by LBC.

When correlated with histopathology, LBC achieved 100% sensitivity and 100% negative predictive value (NPV), compared to 84.2% sensitivity and 98.9% NPV for CPS. This indicates that LBC is more reliable in minimizing false negatives, thereby reducing the risk of missed precancerous and cancerous lesions. Furthermore, LBC reduced the unsatisfactory smear rate (2.67% vs. 6.67% in CPS), ensuring better sample quality and reproducibility.

In comparison with earlier studies, the findings of the present research align with international evidence that LBC provides improved sensitivity and comparable specificity to CPS, with enhanced detection of high-grade squamous and glandular lesions [12,17–22].

Although CPS remains valuable in resource-limited settings due to its low cost and accessibility, the results of this study strongly support the implementation of LBC as the preferred method for cervical cancer screening, especially in high-risk populations. Integration of LBC with HPV testing could further strengthen screening programs, ensuring earlier detection and timely intervention [13,14].

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