



Original Research Article

A comparative study of conventional pap smear with liquid based cytology for early diagnosis of cervical cancer

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ABSTRACT

Introduction: Cervical cancer is the third most common female malignancies worldwide with high mortality. Since the introduction of conventional PAP smear (CPS) mortality from cervical cancer has reduced considerably. Evidence shows that alternative method liquid based cytology (LBC) is more sensitive and accurate for the detection of both squamous and glandular lesions of the cervix.

Objective: The main objective of this study is to compare conventional PAP smear and liquid based cytology and observe for morphologic features, sensitivity, specificity and positive predictive values.

Materials and Methods: This was a prospective study conducted on 240 cases and samples were processed for CPS and LBC. Cytobrush was used for LBC which was suspended and detached in the preservative fluid and processed by manual method. Histopathological correlation was done in 32 cases and HPV DNA testing was done in 76 cases.

Results: LBC showed higher specimen adequacy, cellularity, clean background and uniform distribution of cells than CPS. Overall sensitivity of LBC (83.33%) and negative predictive value was more in LBC (92.85%) as compared to CPS.

Conclusion: In this study LBC showed improved specimen adequacy, better cytomorphological features and higher detection of epithelial cell abnormality. Thus LBC has higher detection rate of precancerous lesion than CPS.

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1. Introduction

Cervical cancer is found to be third most common malignancy affecting women worldwide. A devastating disease with an estimated 20 million cases by the year 2020.¹ More than 85% cases and 88% deaths from cervical cancer occur in developing countries, where women often lack access to cervical cancer screening and treatment. The magnitude of the problem of cancer in the Indian Sub-Continent is alarming² and accounts for one-fourth of the global cervical cancer burden.³

Cervical screening helps in early detection of cancer and thus reducing cancer deaths.⁴ The primary screening test for cervical cancer the Papanicolaou (Pap) smear is responsible

for diagnosing cancerous and precancerous lesions. It is a convenient, cost-effective, simple test and performing this test is considered as a health promotion behaviour.⁵

Pap test has high specificity of 98-99 % and sensitivity ranges from 50-75%.^{6,7} This led to drastic reduction in the incidence of cervical carcinoma. But the Pap test had a high false negative rate due to sampling errors, presence of blood or mucus (obscuring) material, screening errors and interpretation errors.

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. LBC

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gives standardized slides containing a monolayer of well stained well preserved cells which is easier to interpret than the conventional smears. So the aim of this study is to compare the results of conventional PAP smear (CPS) and liquid based preparation and to correlate the results with the histopathological findings.

1.1. Ethical clearance

The study protocol was approved and ethical clearance taken from the University ethics committee.

2. Materials and Methods

This prospective study was conducted on 240 patients selected randomly in the Department of Pathology from December 2016 to September 2018. In this study we had proposed to conduct a comparative analysis of cervical cytology based on result, predictive value by using conventional Pap smear with manual method based liquid based cytology. Women between the ages of 21 to 65 years, women presenting with white discharge per vaginum, post coital bleeding or irregular bleeding, unhealthy cervix on speculum examination and pain in abdomen were included in the study. Non co-operative patients, patients who do not give consent, women who had undergone hysterectomy with removal of cervix, patients with massive bleeding per vaginum, Pregnant women and treated cervical carcinoma cases were excluded from the study.

After obtaining proper consent, detailed history was taken and physical examination was done. Specimens were first obtained for Conventional PAP, Ayre's spatula was inserted into the cervix and gently rotated at 360°. Sample was smeared onto a grease free slide, fixed in alcohol stained with rapid PAP stain.

For Liquid based cytology, manual LBC method was followed. The 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 is taken to the lab where it is again shaken with the vortex to obtain a homogenous mixture. This 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 µl of the suspensions was taken and placed over the slide in a circular manner. The slides are dried and stained with conventional pap stain.

2.1. For histopathological examination

All specimen received in the department following surgery were kept for overnight fixation in 10% neutral

buffered formalin. Grossing was performed according to College of American Pathologist (CAP) protocol. The tissue bits given were processed overnight using a Leica TP1020 Semi-Enclosed B enchtop Tissue Processor (Leica Biosystem, Wetzlar, Germany). The sections were stained with hematoxylin and Eosin on a Leica ST 5010 Autostainer XL (Leica Biosystems, Wetzlar, Germany).

2.2. HPV DNA

Wherever possible, ancillary techniques were applied and 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.

2.3. Statistical analysis

The data was entered into Microsoft Excel 2016 worksheets and then further statistical analysis was performed on IBM SPSS version 23.

3. Results

Most of the patients in this study were in fifth decade. Minimum number of cases (n=22, 9.2%), belonged to more than 60 years age group and maximum number of cases (n=100, 41.7%) belonged to 40-49 age group. Cases with 2 children were 40.4% and 3 children were 42.5% which also represented most number of cases with epithelial cell abnormality.

Women presented with complaints of white discharge per vaginum (19%), bleeding per vagina (15%), dysfunctional uterine bleeding (2.9%), pain in abdomen (4.6%), prolapse (6.7%), burning micturition (0.8%) or came for routine checkup (50.8%). LBC detected abnormality of 24 cases (10%) and CPS detected abnormality of 22 cases (9.1%). In CPS 92% cases were satisfactory while 95% cases were satisfactory in LBC.

Cytomorphological features are compared between CPS and LBC in Table 1. Statistically significant P value was seen in cellularity, uniform distribution of cells, clean background, artefact cellular overlapping and cellular change. Overall sensitivity of PAP was 71% and LBC 83.33% while specificity 93.33% and 87.5% respectively. Positive predictive value of PAP was 83.33% while it was 71.42% in LBC. Negative predictive value of PAP was 87.5% and LBC was 93.33%. The difference in interpretation of results by the two methods were statistically significant and is shown in Table 2.

Table 3 shows histopathological examination findings in 32 cases. Out of the 12 cases of NILM cases in PAP 8 cases had chronic cervicitis and 1 case was of serous borderline carcinoma ovary in HPE. Similarly comparison between HPE and LBC showed out of 18 cases of NILM in PAP, 10 cases had chronic cervicitis, 2 case of prolapse, 2 case of

Table 1: Summary of comparison of LBC and PAP on cytomorphological features

Cytomorphological studies	PAP	LBC
Cellularity	223(93%)	231(96.25%)
Clean background	10(4.2%)	216(90%)
Uniform distribution	14(5.8%)	166(69.2%)
Cellular overlapping	208(86%)	80(33.3%)
Architectural change	205(85.4%)	30(12.5%)
Cellular change	201(83.8%)	35(14.6%)
Nuclear change	33(13.8%)	47(19.6%)
Inflammatory cells	92.2%	22.6%

Table 2: Interpretation of results between conventional PAP smear and LBC

Diagnosis	PAP	LBC	χ^2	DF	P-value
NILM	103 (42.9%)	135 (56.25%)	2407.51	144	<0.001
Inflammatory smear	59 (24.9%)	35 (14.6%)			
Atrophic smear	19 (7.9%)	18 (7.5%)			
Bacterial vaginosis	14 (5.8)	14 (5.8%)			
Candidiasis	4 (1.7%)	4 (1.7%)			
ASCUS	7 (2.9%)	8 (3.33%)			
ASC-H	1 (0.4%)	1 (0.4%)			
HSIL	6 (2.5%)	5 (2.08%)			
LSIL	1 (0.4%)	1 (0.4%)			
SCC	5 (2.1%)	6 (2.5%)			
Adenocarcinoma	1 (0.4%)	1 (0.4%)			
AGC NOS	1 (0.4%)	1 (0.4%)			
Unsatisfactory	19 (7.9%)	11 (4.6%)			
Total	240	240			

papillary endocervicitis, 1 case of necrotising granulomatous lesion and 1 case was of serous borderline carcinoma ovary in HPE.

Table 4 shows Comparison of cytological findings and HPV DNA molecular findings of cytobrush samples done in 76 cases along with positive and negative predictive value. Out of which 10 cases were PCR positive. 6 cases of cytological positive samples were cases of HSIL, ASCUS and 70 cases were cytological negative samples which included NILM, inflammatory smear, bacterial vaginosis and atrophic smear. Out of 6 cytological positive cases 2 were PCR positive and 4 were PCR negative. Whereas out of 70 cytological negative cases 8 cases were PCR positive and 62 cases were PCR negative.

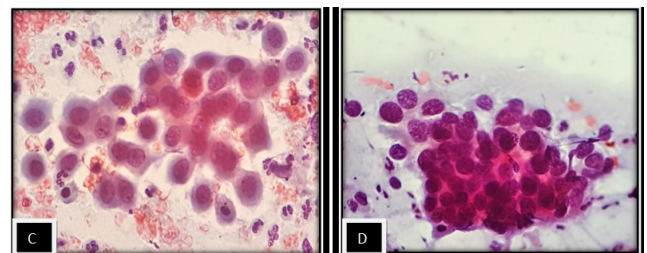


Fig. 2: (C) HSIL in LBC(40X) showing atypical cells in clusters.(D)LSIL- LBC (40X) showing intermediate epithelial cells with nuclear enlargement and hyperchromasia

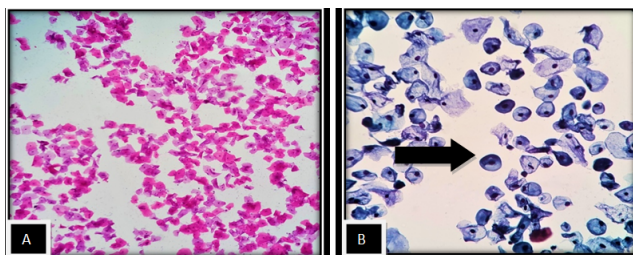


Fig. 1: (A)LBC showing monolayer of cells in a clean background (10X).(B) Atrophic smear showing numerous parabasal cells in LBC(40x)

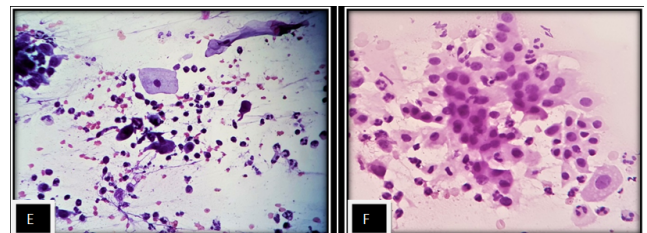


Fig. 3: (E) ASCUS –LBC (40X) showing atypical squamous cells with hyperchromatic nucleus(arrow). (F) ASC-H in LBC(40X)

Table 3: Results of HPE

Diagnosis	HPE	Percentage
Chronic cervicitis	16	50%
Prolapse	2	6.25%
Endocervical polyp	3	9.4%
Papillary endocervicitis	2	6.25%
Necrotising granulomatous inflammation	1	3.1%
Carcinoma	8	25%
Total	32	100

Table 4: Comparison of cytological findings and HPV DNA molecular findings of cytobrush samples.

Total sample	PCR positive samples	PCR negative samples	Positive predictive value	Negative predictive value
Cytological positive cytobrush samples (n=6)	02	04	33.4%	88.6%
Cytological negative cytobrush samples (n=70)	08	62		

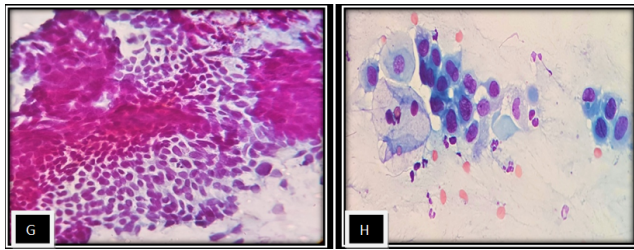


Fig. 4: (G) SCC in PAP(10X) showing sheets of pleomorphic hyperchromatic nuclei. (H) SCC -LBC(40X). Syncytial cluster of tumour cells seen with hyperchromatic nuclei

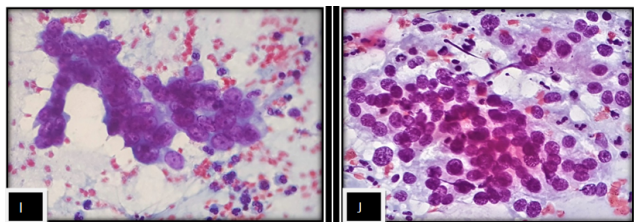


Fig. 5: (I) Adenosquamous Carcinoma in PAP(40X) showing papilloroid fragment of large tumor cells with anisonucleosis and hyperchromatic nucleus. (J) Adenosquamous carcinoma in LBC(40X)

4. Discussion

Widespread cervical screening has reduced the incidence of cervical cancer by more than 50%.⁸ Clarity of microscopic features, small area to be screened, decreased unsatisfactory results, improved sample processing along with the potential for performing additional tests HPV DNA testing makes LBC more promising and acceptable among gynecologist, colposcopists and pathologists.

Out of 240 cases analysed, age of presentation in most cases (41.7%) were seen in the fifth decade of life similar to Chinaka C et al⁹ (31.33%) and Taylor S et al¹⁰ which

also showed maximum number of cases in 40-49 age group. Parity 3 showed maximum number of cases (42.5%) in the present study similar to Khamankar S et al¹¹ (36.7%) while Budak M et al¹² & Macharia H C et al¹³ showed most cases with parity 2. WDPV as a presenting complaint was also seen in studies done by Khamankar S et al,¹³ Sharma J et al,¹⁴ Sherwani RK et al¹⁵ and Singh A et al.¹⁶

This study showed more satisfactory smears in LBC(95%) than in CPS (92%) similar to Hegde V et al¹⁷ (PAP34% LBC67%), Sherwani R K et al¹⁵ (PAP31.9% LBC83.1%), Chinnaka C et al⁹ (PAP53.3% LBC83.3%), Dhananjaya C¹⁸ (PAP84% LBC86%), Singh A et al¹⁶ (PAP78.8% LBC92.5%) & Sharma J et al¹⁴ (PAP92% LBC93%). Contrary to this finding, studies by Longatto-Filho A et al¹⁹ (PAP34% LBC66%) and Ilter E et al²⁰ (PAP34% LBC66%) showed more satisfactory smears in PAP compared to LBC. The present study showed clean background, uniform distribution of cell more in LBC as compared to CPS similar to Hegde V et al,¹⁷ Dhananjaya C et al¹⁸ and Nandini N M et al²¹ According to Uttagawa M L et al²² LBC presented clear background in relation to CPS.

Cellular overlapping, architectural change and cellular morphological change in our study was more in CPS than LBC similar to Kavatkhar et al,²³ Nandini N M et al²¹ and Hegde et al.¹⁷ Strander et al²⁴ and Kirschner B et al²⁵ also showed more number of unsatisfactory smears in CPS than LBC. Cases diagnosed with NILM were more in LBC (55.4%) as compared to CPS (42.9%) in this study similar to Singh V B et al,²⁶ Singh U et al²⁷ and Dhananjaya C et al.¹⁸ However studies done by Stabile S et al²⁸ and Beerman H et al²⁹ reported more number of NILM cases in CPS as compared to LBC. LSIL cases were equal in PAP and LBC in the present study and study done by Sherwani et al.¹⁵ While studies done by Budak et al,¹² Beerman et al,²⁹ Stabile S et al,²⁸ Strander et al,²⁴ Longatto-Filho A et al¹⁹ and Singh U et al²⁷ observed more cases of LSIL in LBC as compared to CPS.

The number of cases diagnosed with HSIL was more in LBC as compared to CS in studies conducted by Almonte M et al,³⁰ Chinaka C et al,⁹ Sherwani R K et al,¹⁵ Beerman H et al,²⁹ Uttagawa M et al²² and Singh A et al¹⁶ which was in concordance with the present study. Studies done by Stabile S et al²⁸ and Uttagawa M et al²² which showed more cases diagnosed with ASCUS in LBC than in CS similar to the present study.

Studies conducted by Budak M et al¹² and Qureshi S et al³¹ reported more cases of SCC in LBC than in CPS. Studies done by Budak M et al¹² and Stabile S et al²⁸ reported more cases of AGS-NOS in LBC as compared to CPS. Singh A et al,¹⁷ Singh V B et al²⁶ and present study reported same number of adenocarcinoma cases in CPS and LBC .

LBC showed better performance and diagnostic accuracy than CPS. Similarly according to study conducted by Sykes P H et al^{30,32} 11 women diagnosed as micro invasive carcinoma was cytologically diagnosed as ASC-H and 9 case of histologically diagnosed and confirmed as ACIS were CIN2/3. Study conducted by Singh U et al²⁸ also showed better diagnostic accuracy for LBC (97.6%) as compared to CPS (79.16 %) with respect to HPE diagnosis. Similarly study by Stabile S A et al²⁸ found that LBC had better concurrence to diagnose atypical cells and the cyto histological concordance was more in LBC than in CPS.

5. Conclusion

CPS is a simple cost effective tool for cervical screening. Manual LBC overcomes the high false positive and false negative results of CPS and also can be further used for ancillary testing such as HPV DNA testing. In this study LBC by manual method showed improved specimen adequacy, better cytomorphological features and better detection of epithelial cell abnormality. The advantage of LBC over CPS was observed in reducing unsatisfactory smears for evaluation and increasing the sensitivity and negative predictive value of the test. The manual LBC has an edge over the automated LBC Thinprep and Surepath methods in cost effectiveness.

6. Disclosure

This study was done as part of thesis from Yenepoya Medical college, Mangalore, 575018 Karnataka

7. Source of Funding

None.

8. Conflict of Interest

None.

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