

A Comparative Study of Manual Liquid-Based Cytology with the Conventional Pap Smear for Cervical Cancer Screening in a Tertiary Care Hospital of Mandya, Karnataka

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ABSTRACT

BACKGROUND

A long pathological process for investigation of precursor lesion squamous intraepithelial lesion (SIL) leads to invasive cervical cancer. This SIL can be detected much earlier before the lesion progresses to invasive cancer. For greater than fifty years, screening for cancer cervix was done by conventional scrape smears and stained by Papanicolaou [Pap] stain but conventional Pap smears (CPAP) have been reported to have low sensitivity. To overcome these drawbacks manual liquid-based cytology [MLBC] was introduced. The objective of this study was to screen females for cervical cancer using CPAP and MLBC techniques and compare the results of these techniques.

METHODS

Cervical cytology samples were obtained from 120 non-pregnant females through specialised Uprep cytobrush with a detachable head. Thus, obtained samples were first smeared onto a clean glass slide for CPAP smear, and the whole head to cytobrush was dropped into the specialised Uprep liquid preservative medium and processed by using Uprep Cytospin machine to obtain MLBC smears. Both the smears were stained by conventional Pap stain and reported according to 2014 Bethesda system.

RESULTS

In this study, the CPAP method had a greater number of unsatisfactory smears than that of MLBC method which was statistically significant. MLBC identified more number of intraepithelial lesions when compared to CPAP and MLBC had an increased detection rate [IDR] of 73.68 % over CPAP.

CONCLUSIONS

Analysis of our results showed that MLBC had more advantages over CPAP. Since the cost effective MLBC has an improved rate of detection of abnormal lesions, MLBC can be used as a routine technique for screening of cancer cervix in India. Also, MLBC offers an important advantage of performing both human papillomaviruses deoxyribonucleic acid (HPV DNA) test and cytological analysis on a single sample.

KEYWORDS

Cervical Cancer, Conventional Pap Smear, Manual Liquid Based Cytology, Human Papilloma Virus (HPV DNA), Bethesda System

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BACKGROUND

Uterine cervical cancer is a global health problem. Cancer cervix is second most common cancer in India among women aged between 15 and 44 years and is responsible for 67,477 deaths every year.¹ Cervical cancer epidemiology shows wide geographical variation in its occurrence, these differences are related to persistent infections by high-risk human papilloma virus (HPV) especially type 16 and 18 which leads to development of cancer cervix.² In India about 5 % of women harbour cervical HPV - 16 / 18 at a given time, and 83.2 % of invasive cervical cancers are caused due to infection by HPV 16 / 18. Invasive cervical cancer begins with a precursor lesion called squamous intraepithelial lesions (SIL) which can be detected years before development of invasive carcinoma.³ In 1988, at a meeting in Bethesda, SIL was classified into three main categories: ASCUS, LSIL and HSIL.⁴ Atypical squamous cells of undermined significance (ASCUS), low grade squamous intraepithelial lesion (LSIL) which corresponds to HPV associated mild dysplasia and cervical intraepithelial neoplasia (CIN I) and high grade squamous intraepithelial lesion (HSIL) which corresponds to moderate and severe dysplasia, carcinoma in situ (CIS) and CIN II and CIN III.

Conventional Pap (CPAP) smears have been the wall for screening of cancer cervix since 1960. CPAP is accredited in many developed countries for successfully reducing the incidence and mortality of invasive cervical cancer, but the accuracy of this tool is controversial due to low sensitivity.^{5,6} Liquid based cytology (LBC) was introduced as an alternative to CPAP in mid 1990s. LBC increased the detection rate of HSIL, reduced the number of unsatisfactory and "satisfactory-but-limited-by" specimens and provided residual cellular material for further molecular testing (detection of HPV DNA) which were the major advantages of LBC over CPAP.⁵ ThinPrep (cytyc corp²) and SurePath (TriPath Imaging²) are two Food and Drug Administration (FDA) approved liquid-based preparations to obtain LBC smears. But in this study, a relatively inexpensive manual method of LBC (MLBC) by using cytospin to make direct smears was used to improve the screening method for cervical cancer in our tertiary care hospital of Mandya district.

Objectives

This study was conducted to screen females for cervical cancer using the conventional Pap smears and report them according to 2014 Bethesda System of Classification, screen females for cervical cancer using the manual liquid-based preparation, report them according to 2014 Bethesda system of classification and compare results of conventional Pap smear cytology with manual liquid-based cytology.

METHODS

The present study was a cross sectional study undertaken during the period of January 2017 to June 2018 where

cervical cytology samples were obtained from a total of 120 subjects aged between 20 - 70 years who were patients attending the Obstetrics and Gynaecology [OBG] Outpatient Department of Mandya Institute of Medical Sciences [MIMS]. Both conventional Pap smears and manual liquid based cytology smears were obtained from a single sample collected from each patient.

Inclusion Criteria

Cervical cytology samples were obtained from all women attending the OBG outpatient department of MIMS aged between 20 - 70 years with presenting complaints of post-menopausal bleeding / spotting, white discharge per vagina, post-coital bleeding, irregular menstruation and from women who have visited for cervical cancer screening.

Exclusion Criteria

- Pregnant women.
- Patients having massive bleeding per vagina.
- Treated cases for cervical cancer.

Women who has undergone hysterectomy

Sample Size Calculation

Sample size was calculated using sensitivity formula.

$$\begin{aligned} \text{Sample size (N)} &= Z^2_{1-\alpha/2} \times S_N \times (1 - S_N) / L^2 \\ &\times \text{Prevalence} \end{aligned}$$

S_N – Sensitivity of Pap smear – 86 %⁷ Prevalence – 0.8,⁸ L – Absolute precision – 0.07, Z – 1.96

$$N = 3.84 \times 0.86 \times (1 - 0.86) / (0.07)^2 \times 0.8$$

$$N = 0.462336 / 0.00392$$

$$N = 117.94$$

Sample size (N) \approx 120 samples

Sample Collection

In this study we have utilised the Uprep manual liquid-based technology for studying the cervical cytology. After getting the consent from the patients, detailed clinical history and complete clinical examination of each patient was obtained. A specialised Uprep plastic cytobrush was used to obtain cervical cytology samples. The Uprep cytobrush has two types of bristles, long bristles at the centre and short bristles at the periphery. The main advantage of cytobrush is that after insertion into the cervical os the long bristles are inserted into the endocervical cavity and short bristles are outside the cavity. After the brush was positioned, the Uprep cytobrush was rotated 5 times in clockwise direction for 360⁰ rotations in the same direction. This helped in collection of cells from endocervix by long bristle and from ectocervix and transformation zone from short bristles of the cytobrush. Split sampling technique was used where one side of cytobrush was scraped on a clean glass slide and fixed with 95 % ethyl alcohol for CPAP and the entire detachable head of Uprep cytobrush was detached and dropped into a plastic

preservative bottle containing 14 ml of Uprep methanol based preservative. The bottle was tightly capped and patient's details were noted down on bottle label. After fixation, the CPAP smears were stained by conventional Pap stain and reported according to 2014 Bethesda system of classification.

For MLBC Smears

The following steps were followed for processing of each sample:

1. The sample in Uprep preservative bottle was agitated thoroughly to obtain homogenous mixture of the sample.
2. 7 ml of sample solution was transferred into a centrifuge tube which was pre-filled with 4 ml of Uprep separator solution.
3. Centrifuged at 2000 rotations per minute (rpm) for 5 minutes.
4. Supernatant was discarded and the pellet was added with 1 / 2 drops of distilled water or saline depending on the density of the pallet.
5. The Uprep specialised pre-charged optic coat slide was placed in the Uprep centrifuge holder and the funnel was fitted above the slide after which one drop of distilled water and 50 microlitres of pellet was placed inside the funnel on the glass slide.
6. Centrifuge at 1500 rpm for 2 minutes in Uprep Nanocyt machine was done.
7. The funnel was remove to get a clear round smear and the smear was air dried.
8. These smears were further stained by conventional PAP stain and reported according to 2014 Bethesda system of classification.

Statistical Analysis

The detailed data which was collected from 120 patients was entered in Excel sheet and the data was analysed by trial version of Statistical Package for the Social Sciences (SPSS) for statistical analysis. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for categorical variables. Data was also represented using appropriate diagrams. The association between explanatory variables and categorical outcomes was assessed by cross tabulation and comparison of percentages. Chi-square test was used to test statistical significance. P-value < 0.05 was considered statistically significant.

RESULTS

Present cross-sectional study was conducted in the Department of Pathology, MIMS, Mandya from January 2017 to June 2018, for a period of 18 months on 120 patients. The results of this study were as follows.

The mean age of the study group was 37.87 ± 11.41. Minimum and maximum age of the patients in this study was 20 and 74 years respectively. Majority of patients, 62 (51.70 %) had a chief complaint of white discharge per vagina, 43

(35.80 %) were asymptomatic, 6 (5 %) had irregular bleeding / spotting per vagina and 1 (0.8 %) patient had post-menopausal bleeding.

Out of 120 patients, in MLBC cervical samples were adequate in 117 (97.5 %) patients and inadequate in 3 (2.5 %) patients whereas in CPAP cervical samples were adequate in 113 (94.17 %) patients and inadequate in 7 (5.8 %) patients respectively. This difference in the proportion was statistically significant (P-value 0.040).

Epithelial Cell Abnormalities

In MLBC, 33 (27.5 %) patients were diagnosed with epithelial cell abnormalities whereas in CPAP 19 (15.88 %) patients were diagnosed with epithelial cell abnormalities. MLBC detected ASCUS in 6 (5 %) patients whereas CPAP detected ASCUS in 2 (1.66 %) patients. This difference in the proportion was statistically significant (P-value 0.003) and thus the detection rate of MLBC on epithelial cell abnormality (ASCUS) is relatively more significant than CPAP.

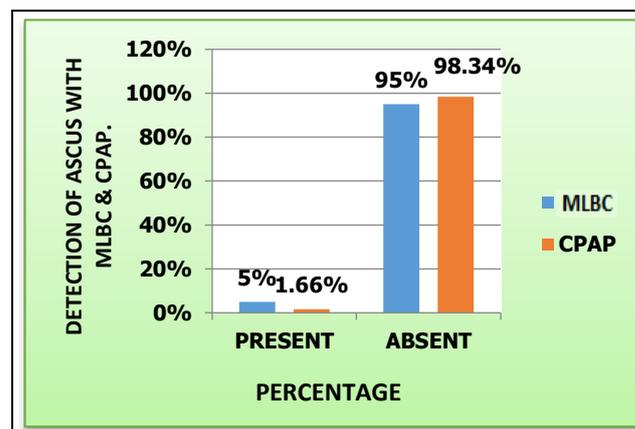


Figure 1. Comparison of Detection of ASCUS

MLBC detected LSIL in 11 (9.1 %) patients whereas CPAP detected LSIL in 7 (5.83 %) patients. This difference in the proportion were statistically significant (P-value 0.001) and thus the detection rate of MLBC on epithelial cell abnormality (LSIL) is relatively more significant than conventional Pap smear method.

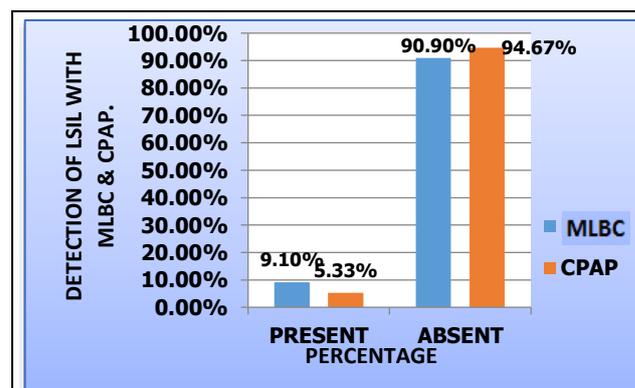


Figure 2. Comparison of Detection of LSIL with MLBC and CPAP (N = 120)

MLBC detected HSIL in 10 (8.33 %) patients whereas CPAP detected HSIL in 4 (3.33 %) patients. This difference in the proportion were statistically significant (P-value < 0.001) and thus detection rate of MLBC on epithelial cell abnormality – HSIL is relatively more significant than CPAP.

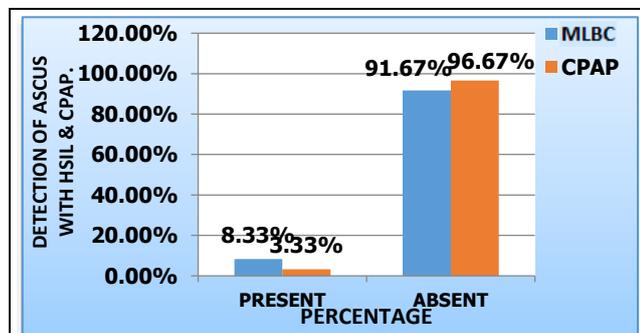


Figure 3. Comparison of Detection of HSIL by MLBC and CPAP (N = 120)

MLBC detected SCC in 4 (3.33 %) patients whereas CPAP detected SCC in 4 (3.33 %) patients thus the detection rate of MLBC on epithelial cell abnormality – SCC is equal to that of CPAP. Both MLBC and CPAP detected glandular cell abnormality in 2 (1.66 %) patients thus the detection rate of MLBC on glandular cell abnormality, not otherwise specified [NOS] is equal to that of the detection rate of conventional PAP smear method.

Morphological Features

The morphological features of cervical cytology cells were well maintained in MLBC when compared with CPAP as MLBC slides had much more cleaner background, uniform distribution of cells, minimal overlapping of cells with presence of less artefacts when compared to CPAP which helped in diagnosing of more epithelial cell abnormalities.

Rate of Concordance & Agreement between MLBC and CPAP

In the current study, there was moderate agreement between the two methods (k = 0.604, P = < 0.001) with rate of concordance [21 + 81 / 120 * 100 = 85 %] being 85 %

| Conventional Diagnosis | MLBC Diagnosis | | Kappa Value | Standard Error | P-Value |
|------------------------|------------------|-----------------|-------------|----------------|---------|
| | Present (N = 25) | Normal (N = 95) | | | |
| Present | 21 (84 %) | 14 (14.74 %) | 0.604 | 0.083 | < 0.001 |
| Normal | 4 (16 %) | 81 (85.26 %) | | | |

Table 1. Comparison of MLBC Diagnosis with Conventional Diagnosis (N = 120)

Increased Detection Rate [IDR] of Abnormal Cervical Cytology Smears

Increased detection rate (IDR) of MLBC was calculated as follows:

$$IDR = ((Pm - Pc) / Pc) * 100; IDR = ((33 - 19)) / 19 * 100; IDR = 73.68 \%$$

Pm is number of positive cases through MLBC and Pc is number of positive cases for CPAP.

MLBC method had an increased detection rate of 73.68 % when compared with that of CPAP.

Predictive Validity of MLBC in Predicting CPAP Diagnosis (N = 120)

MLBC had a sensitivity of 84.00 % (95 % CI 63.92 % to 95.46 %) in predicting CPAP diagnosis with a specificity of 85.26 % (95 CI 76.51 % to 91.70 %), false positive rate of 14.74 % (95 CI 8.30 % to 23.49 %), false negative rate of 16.00 % (95 CI 4.54 % to 36.08 %), positive predictive value of 60.00 % (95 CI 42.11 % to 76.13 %), negative predictive value of 95.29 % (95 CI 88.39 % to 98.70 %), and the total diagnostic accuracy was 85.00 % (95 CI 77.33 % to 90.86 %) in predicting the diagnosis of CPAP.

Predictive Validity of CPAP in Predicting MLBC Diagnosis (N = 120)

CPAP had a sensitivity of 60.00 % (95 % CI 42.11 % to 76.13 %) in predicting MLBC diagnosis, specificity of 95.29 % (95 CI 88.39 % to 98.70 %), false positive rate of 4.71 % (95 CI 1.30 % to 11.61 %), false negative rate of 40.00 % (95 % CI 23.87 % to 57.89 %), positive predictive value of 84.00 % (95 % CI 63.92 % to 95.46 %), negative predictive value of 85.26 % (95 % CI 76.51 % to 91.70 %), and total diagnostic accuracy was 85.00 % (95 % CI 77.33 % to 90.86 %) in predicting the diagnosis of MLBC.

DISCUSSION

For more than 50 years, conventional Pap smear (CPS) has been the mainstay for cervical cancer screening without major changes in the techniques related to preparation and interpretation. Despite its success as a preventive screening tool for cervical cancer, CPS has its limitations (Deshou et al.⁹). False negatives in CPS may be related to inadequate sampling, inadequate transfer of sample onto glass slide where approximately 20 % of the cells collected were smeared on the glass slide in CPS (Sherwani et al.¹⁰ Deshou et al.⁹ Maksem et al.).¹¹

To overcome these problems, a new slide preparation method MLBC was introduced by Maksem et al.^{11,12} Many studies have shown that with proper training, MLBC results in a higher diagnostic yield than CPAP (Baandrup et al.¹³ Sherwani et al.¹⁰). MLBC is a technique which enables cells to be suspended uniformly in a monolayer by centrifuge technique, which improves specimen adequacy and detection of precursor lesions. MLBC is also cost effective which can be used in low resource settings and can be a great alternative to CPAP.¹⁴

In our cross-sectional study, MLBC method was found comparable to CPAP on some parameters and superior on few others which are discussed as follows.

In our study, the unsatisfactory smear rate was reduced from 5.8 % to 2.5 % in MLBC when compared with CPAP method. The most common reason for unsatisfactory smears

was low cellularity in both categories. In CPS, there was higher rate of unsatisfactory smears due to the presence of obscuring materials such as blood, inflammatory cells along with dirty necrotic background which hinders screening process. The reduction of unsatisfactory smears in MLBC samples was consistent with many previous studies. Our finding was in concordance with the studies of Bergeron (2001),¹⁵ Afsan el al. (2007),¹⁶ Strander al. (2007),¹⁷ Beerman et al. (2009),¹⁸ Singh et al. (2015).¹⁹ and Costa (2015).²⁰ They also reported an increased number of satisfactory cases on LBC than conventional smears, which was statistically significant.

| Authors [Satisfactory Smears] | Years | CPS (%) | LBC (%) | Study [Unsatisfactory Smears] | CPS | LBC |
|----------------------------------|-------|---------|---------|----------------------------------|------|------|
| Bergeron et al. ¹⁵ | 2001 | 88.4 | 99.2 | Monsonogo J et al. ²¹ | 9 | 13 |
| Afsanet al. ¹⁶ | 2007 | 31.9 | 83.1 | Taylor S, et al. ²² | 0.8 | 2.2 |
| Stranderet al. ¹⁷ | 2007 | 99.7 | 99.3 | Davey DE et al. ²³ | 1.8 | 3.1 |
| Beermanet al. ¹⁸ | 2009 | 99.11 | 99.87 | Afsan N et al. ¹⁴ | 68.1 | 18.9 |
| Singh et al. ¹⁹ | 2015 | 95.7 | 98.3 | Svkes PH et al. ²⁴ | 9.1 | 2.7 |
| Costa et al. ²⁰ | 2015 | 98.29 | 95.62 | Sharma J et al. ²⁰ | 8 | 7 |
| Stabile et al. ²¹ | 2015 | 98 | 99 | Present study | 16 | 3 |
| Present Study | 2017 | 93.6 | 98.8 | | | |

Table 2. Comparison of Percentage of Satisfactory Smears Obtained by Conventional and LBC by Various Authors

CPS: Conventional Pap Smear, LBC: Liquid-Based Cytology

According to Afaf et al.²² in MLBC smears had marked decrease in artefacts, contaminating mucus, blood and cells are evenly distributed on slides as the centrifugation in MLBC method offers a proper mixing of samples. Likewise, in our study MLBC smears gave more clear results with clear background, less artefacts and lesser degree of cellular overlapping when compared with CPAP, where the smears predominantly had an unclear background with non-uniform cellular distribution and the cells had more of drying artefacts. This finding was similar to studies such as Brud et al.²³ Akbar et al.²⁴ Nandini et al.¹² Celik et al.²⁵ and Afaf et al.²²

The current study reports a higher rate of detection of epithelial abnormalities than CPAP which corresponds to various studies such as:

1. Studies where ASCUS detection was more with MLBC than with CPS [Kavatkar et al.²⁶ Cheung et al.²⁷ Strander et al.¹⁷ Ronco et al.²⁸
2. LSIL detection was more with MLBC than with CPS [Simion et al.²⁹ Nandini et al.¹² Kavatkar et al.²⁶
3. HSIL detection was more with MLBC than with CPS [Austin and Ramzy,³⁰ Bishop et al.³¹ Nandini et al.¹²

In the current study, there was increased detection rate of abnormal cervical cytology smears with MLBC of 73.68 %. However, this was low compared to 150 % as documented in the study done by Nandini et al.¹² and also low as compared to 85.7 % documented in the study conducted by Mutuku Onesmus Muia et al.³²

In the current study, there was moderate agreement between the two methods (k = 0.604, P = < 0.001) with rate of concordance being 85 % which was similar to the various studies by Nandini et al.¹² and Anitha N Kavatkar et al.²⁶ with the rate of concordance being 86 % and 88.8 % respectively.

Hence, the MLBC which we have used in our study is an inexpensive, cost effective method of LBC is superior in adequacy and utility when compared to CPAP.

CONCLUSIONS

LBC is recommended for screening of cancer cervix, but it involves use of automated devices that are high in cost. But the less expensive MLBC which was used in our study was found to be better than conventional Pap as it overcomes various limitations of CPAP and can be used as an alternative strategy for screening of cancer cervix in low resource settings.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.

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