ORIGINAL RESEARCH PAPER

Volume-7 | Issue-4 | April-2018 | PRINT ISSN No 2277 - 8179

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

# A COMPARATIVE ANALYSIS OF MANUAL LIQUID BASED CYTOLOGY VERSUS CONVENTIONAL SMEAR

Pathology

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## ABSTRACT

**Introduction:** Pap smear is the conventional screening procedure for cervical cancer. MLBC (Manual Liquid Based Cytology) has been developed as a cost effective alternative as it has a short screening time, better morphology and background while also providing residual material to test for HPV DNA.

**Aim:** To evaluate the diagnostic efficacy of MLBC and compare the morphological parameters with conventional PAP smear.

Materials and Methods: This is a prospective study comparing 50 cervical smears using Pap smear and MLBC by the split sample method. The smears were stained by Rapid Pap stain and reported using Bethesda System.

**Results:** In MLBC 18 (36%) cases were reported as inadequate smear but none of the smears were inadequate in CS. By MLBC method 10(20%) cases showed inflammatory cells while CS showed inflammatory background in 31(62%) cases. MLBC preparations showed 2 (4%) cases of LSIL while CS showed 2(4%) HSIL and 4(8%) LSIL. The interpretation of results revealed that there was a statistically significant difference between the two methods ($P<0.001$).

**Conclusion:** MLBC is better than CS in that it has uniform distribution, reduced cellular overlapping and a cleanbackground but it was inferior in detecting epithelial lesions. So, by improving the MLBC technique, its efficiency can be increased for use in low resource settings.

## KEYWORDS

Manual Liquid Based Cytology, Pap smear

## INTRODUCTION

Cervical cancer is one of the leading causes of cancer related deaths in women of the developing countries. It is a slow growing tumor and hence can be effectively prevented by screening and early diagnosis. Cervical cytology has proved to be one of the most successful examples of cancer screening in many developed countries and has resulted in significant decrease in incidence and mortality from invasive cancer by detecting and eradicating the pre invasive lesions.[1] Specificity of this test is 98 to 99%. But the sensitivity, ranges from 50%-75% [2][3]. Several limitations of conventional Pap test are identified such as Inadequate transfer of cells to slide, Ununiform distribution of abnormal cells ,Presence of obscuring inflammation, blood and overlapping of epithelial cells.[4]

These limitations were overcome by Liquid based Cytology(LBC). One of the major limiting factor of LBC is high cost. An indigenous method of LBC was developed using fixatives and polymer solutions prepared in one's own laboratory called the Manual Liquid based cytology(MLBC). The present study was undertaken to compare this low cost Manual LBC with conventional Pap smear.

## AIMS AND OBJECTIVES:

1. To evaluate the diagnostic efficacy of Manual Liquid Based Cytology.

2. To make a comparative morphological analysis of Conventional Pap smear with Manual liquid based cytology smear.

## MATERILAS AND METHODS:

This study was conducted in the Department of Pathology at Tirunelveli Medical College from August 2010 to April 2012.A split sample study was done after the approval of the Ethical committee .

In our study we conducted a comparative analysis of cervical cytology by using conventional Pap smear with manual liquid based cytology .Samples were collected from the patients attending the Gynaecology Outpatient Department after obtaining consent. The patients presenting with white discharge, post menopausal bleeding, unhealthy cervix on speculum examination were included in our study . 50 cases were subjected to comparative analysis of manual liquid based cytology with Conventional Pap smear.

For MLBC fixative and cell base were prepared in our laboratory. Fixative was composed of Absolute alcohol , Glacial acetic acid and 10% formalin . The Cell Base is used to suspend the cells in monolayer sheets. The cell base was prepared by using Agarose (1 gram of Agarose was added to 75 ml of deiodinized water in a beaker and mixed to an even suspension .The suspension is then boiled, until a yellow colour clear suspension is obtained.),Poly Ethylene Glycol (PEG) and Absolute alcohol in appropriate quantity.

## PREPARATION OF SLIDES

For MLBC Samples are collected by using wooden spatula .The spatula was inserted into cervical canal and rotated to 360 degrees. The head of the spatula is broken into a vial containing 4 ml of fixative and fixed for 1-4 hours. After fixation samples were mixed thoroughly to obtain a homogenous mixture. This mixture was then centrifuged at 800 rpm for 10 min. The supernatant was discarded and 1-2 ml of Base polymer solution was added. This was further mixed thoroughly to obtain a homogenous suspension. 2 drops of suspension was pipetted and placed over a glass slide and spread in a circular manner. The slides were then air dried and stained with Rapid pap stain.

The conventional smears were stained by Rapid Pap stain.

The cellularity of the smears were assessed and compared by both MLBC and Conventional Method. Smears were assessed for cellularity by grading into 3 grades (1, 2, 3) based on the number of cells in each 40X field.

GRADE 1-up to 150 cells –inadequate for reporting
GRADE 2-150-500 cells-just adequate
GRADE 3-> 500 Cells -Adequate

The other parameters of comparison were Clean background, Uniform distribution of cells, cell overlapping, inflammatory cell background, cytoplasmic distortion and nuclear irregularity.

## OBSERVATION AND RESULTS

The smears were studied by using 7 morphological parameters such as cellularity , clean background, uniform distribution, cell overlapping, inflammatory background, nuclear distortion, cytoplasmic distortion and interpretation of results. For reporting, The Bethesda system 2001 was used in both methods.

The data regarding Conventional smears vs Manual Liquid based cytology were compared and interpreted by $\chi 2$ (Chi- square) test. The above procedure of statistical analysis and interpretations were made by the statistical software IBM SPSS statistics 20. The P-values <0.05 (P<0.05) were treated as significant.(TABLE 1 & 2)

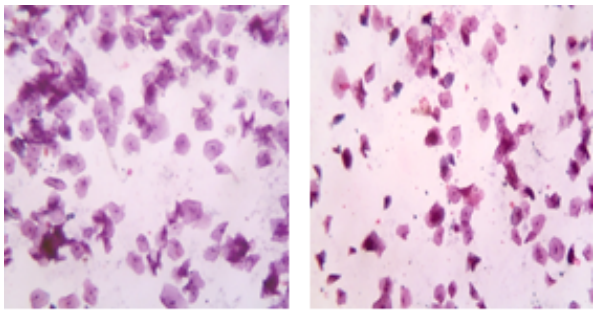


**FIG 1-MLBC- Reduced Cellular overlapping & reduced inflammatory cells, Mucus in the background (100X)**

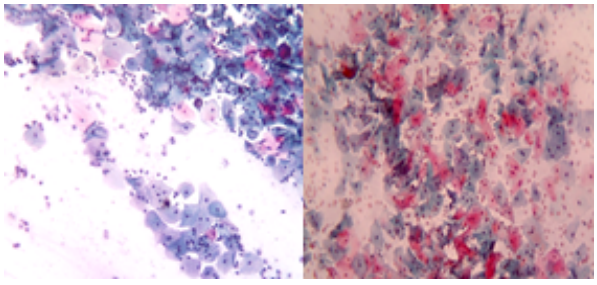


**FIG 2-CS-Cellular overlapping & Excessive inflammatory cells, Mucus in the background (100X)**

**TABLE 1:STATISTICAL ANALYSIS OF CS AND MLBC RESULTS**

| Category | Results | MLBC n=50 | n=50 | | CS | $X^2$ | DF | P |
|---|---|---|---|---|---|---|---|---|
| | | No | % | No | % | | | |
| Cellularity | Grade 1 | 18 | 36 | 0 | 0 | 49.613 | 2 | P<0.001 |
| | Grade 2 | 25 | 50 | 9 | 18 | | | |
| | Grade 3 | 7 | 14 | 41 | 82 | | | |
| Clean background | Present | 29 | 58 | 3 | 6 | 31.066 | 1 | P<0.001 |
| | Absent | 21 | 42 | 47 | 94 | | | |
| Uniform distribution | Present | 12 | 24 | 7 | 14 | 1.624 | 1 | P>0.05 |
| | Absent | 38 | 76 | 43 | 86 | | | |
| Cell overlapping | Present | 33 | 66 | 43 | 86 | 5.482 | 1 | P <0.05 |
| | Absent | 17 | 34 | 7 | 14 | | | |
| 0bscuring Inflammatory cell | Present | 10 | 20 | 34 | 68 | 14.42 | 1 | P<.0.00 1 |
| | Absent | 40 | 80 | 16 | 32 | | | |
| Cytoplasmic distortion | Present | 24 | 48 | 6 | 12 | 15.439 | 1 | P<.0.00 1 |
| | Absent | 26 | 52 | 44 | 88 | | | |
| Nuclear distortion | Present | 15 | 30 | 5 | 10 | 6.250 | 1 | P<0.01 |
| | Absent | 35 | 70 | 45 | 90 | | | |

**TABLE-2 : INTERPRETATION OF RESULTS BETWEEN THE TWO PROCEDURES.**

| INTERPRETATION | CS | MLBC | TOTAL | X2 | DF | P |
|---|---|---|---|---|---|---|
| Inadequate | 0(0%) | 18(36%) | 18 | 71.9 | 5 | P<0.001 |
| NILM | 9(18%) | 20(40%) | 29 | | | |
| NILM-IS | 31(62%) | 10(20%) | 41 | | | |
| ASCUS | 4(8%) | 0(0%) | 4 | | | |
| LSIL | 4(8%) | 2(4%) | 6 | | | |
| HSIL | 2(4%) | 0(0%) | 2 | | | |
| Total | 50 | 50 | 100 | | | |

**DISCUSSION:**

The best prevention programs should be determined regionally on the basis of local resources and acceptability.Liquid based cytology is an alternative method to conventional pap smears to overcome its limitations.One of the major limitations of LBC method compared to CS is its higher cost. To overcome this, few studies have been done on manual liquid based cytology technique.

In 2001 Maksem et al reported on the formulation of an alcohol-agar solution as cell base for manual slide preparation. This inexpensive method is based on Saccomano's technique for sputum processing. The difference of the MLBC from Saccomano's techniques involves substitution of vortex mixer for a mechanical blender and addition of nutrient agar, glycerin and linear alcohol alkoxylate to a PEG-alcohol solution to produce a monolayer sheet. [5].He found that only 0.2% of smears are unsatisfactory which was solely attributed to inadequate sampling. He also noted that there was 3 fold increase in the detection of SIL & 45% reduction of ASCUS diagnosis compared to previous year statistics.

In 2005 Maksem et al again reported a technical improvement in MLBC method. An improved polymer-Gel solution was prepared by using DNA –grade agarose, PEG and 0.1% poly-L-lysine solution which can be stable for 2 years.In his study he also found that most of the discrepancies between Automated LBC & MLBC method may be related to the size of the screened area .[6]

To overcome this disadvantage in 2006 Lee et al conducted a split sample study. In his study, the cells suspended in polymer solution were spread over the slide to cover a circular area of 20-25 mm in diameter which was air dried and stained with Pap stain. He noted that there was 76.3% overall agreement between MLBC & CS.[7]

Anita N Kavatkar et al [8] and NM Nandini et al [9] in their study prepared cervical cytology smears using the manual LBC method. The samples were fixed in a fixative prepared in their own laboratory by using alcohol, water, sodium chloride &10% formalin. The cell base was prepared by using agarose, polyethylene glycol, alcohol, and poly-L-Lysin. They found that MLBC method was comparable to conventional smears.

In our study we adopted the same method of Kavatkar et al for preparation of fixative ,cell base & compared the morphological features of the both preparation .

In our study, intact membrane of polymer solution that hold the cells to the slide was observed in 64% of cases. This is an indicator of good processing technique. A study conducted by Kavatkar et al showed intact membrane in 97/105 (92%) cases [8]Our study showed satisfactory (adequate cellularity) smear in more number of cases of CS(82%) than MLBC (64%). Whereas in study conducted by NM Nandini et al more number of satisfactory smears were obtained in MLBC (99%). 9

In our study, 29(58%) cases revealed clean back ground in MLBC compared to 3(6%) cases in CS with clean background which was statistically significant (P<0.001).The study by Chinmayee et al (34%cases) [10] and NM Nandini et al (100%) also showed similar results .

In our study uniform distribution of cells were found in 24% of MLBC preparations and 14% of conventional smears[FIG 1& 2]. A study by NM Nandini et al showed uniform distribution of cells in most of the cases of MLBC compared to CS.[9]

Our study showed cellular overlapping in more number of CS (86%) than MLBC preparations(66%) which is similar to the study by Nandini et al [9] &Kavatkar et al .[8]

In our study Inflammatory cells were seen in 20% of the cases of MLBC smears whereas 68% cases of CS showed inflammatory background. From our study we found that even though the inflammatory cells were present in MLBC smears, it was not obscuring the epithelial cell morphology. This correlates with the study by Kavatkar et al.In the study by Chinmayee et al, inflammatory background was seen more in the conventional smears 68.04% compared to MLBC with 62.9% [10]. Another study by NM Nandini et al showed that inflammatory infiltrates were observed in less number of MLBC (20%) cases compared to CS (42%)cases.[9]

In our study 36%(18) of cases were reported as inadequate smears in MLBC ,due to scant cellularity and 40% (20 cases) were reported as Negative for intraepithelial lesions or malignancy(NILM). Two cases of NILM on MLBC were reported as HSIL in CS. These slides on CS showed predominantly normal squamous cells with a few clusters

showing high grade intraepithelial lesions. We found Four cases of LSIL in CS in MLBC only two cases were identified. 3 cases of inadequate smears and one case of NILM on MLBC were reported as ASCUS on CS. This revealed that there was a statistically significant difference in the interpretation of results between the two methods . In the study by Chinmayee et al, 93% of high grade readings in pap smears corresponded with high grade lesions compared with 83% for MLBC. [10] A study by NM Nandini et al on comparing the interpretation of smears showed same number of normal smears in both methods. But diagnosis of Low Grade squamous intra epithelial lesion (36%) was more by MLBC method.[9]A study by Kavatkar et al showed that there was an 88.8% agreement in the diagnosis by both methods.[8]

In our study MLBC smears were not able to pick up these cases with intraepithelial lesions, mostly due to less cellularity. This might possibly be due to the split sample method adopted which resulted in scant cellularity which might probably be ruled out by direct sampling methods. Austin et al (1998)found endocervical components more in CS than MLBC, which has been attributed to the split sample collection protocol and this can be overcome by direct sampling method.[11] Direct sampling method for MLBC could have detected more intraepithelial lesions

## SUMMARY AND CONCLUSION

This study was conducted to evaluate the efficiency of Manual liquid based cytology which is a cost effective version of Liquid based Cytology and also to compare its morphological characteristics with Conventional smears.

The conventional cytology is a sensitive method of cervical screening and it detected all cases of intraepithelial lesions in our study. Manual liquid based cytology method provides cytology smears with clean background that have significantly lesser obscuring inflammatory cells or mucus in the slides. But still the percentage of satisfactory smears is less compared to CS .Most of the intraepithelial lesions of cervix were not detected by our MLBC method. This has been due to reduced cellularity in some cases or non-representation of abnormal cells in other cases. This concludes that though MLBC is comparable to conventional smear in some aspects and inferior to it in other aspects, it can be improved and standardized to yield better results. Direct sampling instead of split sampling will increase cellularity and the other advantages are that it includes preservation of specimen for ancillary studies like HPV DNA detection, better morphology and cleaner background and hence better analysis of nuclear morphology. Thus it is worthwhile to further work on MLBC for improving sensitivity for use in resource poor settings as an alternative to other automated liquid based methods.

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