ORIGINAL RESEARCH PAPER
Volume - 11 | Issue - 09 | September - 2022 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DIAGNOSTIC ROLE OF MANUAL METHOD OF LIQUID BASED CERVICAL CYTOLOGY

Pathology

Dr Rajatha Anand* Assistant Professor, Department of Pathology, Sri Siddhartha Medical College, Tumkur, Karnataka *Corresponding Author

Dr Geetha JP Professor, Department of Pathology, Sri Siddhartha Medical College, Tumkur, Karnataka

ABSTRACT

Background: The introduction of the Pap test as a screening procedure to detect precancerous lesions has helped to reduce both the incidence and mortality of cervical cancer. In order to improve the sensitivity, specificity of the Pap test there has been a shift from conventional cytology to Liquid based cytology. A disadvantage of the Liquid based cytology is the high cost automated processing equipment. On the other hand, Manual Liquid-Based Cytology is a technique that also enables cells to be suspended in a monolayer similar to Liquid based cytology and improves detection of precursor lesions, specimen adequacy and also a cost effective strategy in limited resource setting. **Objective:** To compare the diagnostic utility of Manual liquid-based cytology versus Conventional pap smear in cervical cancer detection on cervical smears. **Materials and Methods:** In this prospective comparative study, 210 samples were collected for conventional pap smears and Manual Liquid-Based Cytology to compare the two methods and to correlate with histopathological diagnosis where specimen was available. **Results:** The Manual Liquid-Based Cytology showed sensitivity of 100% , specificity of 91.7% while conventional smears had sensitivity of 75% and specificity of 85.7%.The diagnostic accuracy of Manual Liquid-Based Cytology was 98% and that of conventional method was 78% The p value was less than 0.005 in Manual Liquid-Based Cytology (0.0001) and CPS (0.0001) methods considering histopathology as gold standard. **Conclusion:** This study showed that Manual Liquid-Based Cytology has a higher sensitivity and specificity than conventional pap smears.

KEYWORDS

Cervical cytology, Conventional Pap smear, Manual liquid based cytology.

INTRODUCTION

Cervical cancer is an issue of foremost importance globally, specifically affecting the developing nations [1]. Cervical cancer is the third common cancer in the world in females and second leading cancer for death in women [2]. The burden of cervical cancer in India is enormous, and it continues to remain a major public health problem for women in India, afflicting Indian women-physically, psychologically, socially and financially [3, 4]The introduction of the Pap test in the 20th century witnessed a remarkable decline in the mortality from cervical cancer in many developed countries and reduced the incidence of cervical cancer especially in developing countries. However the Pap smears has been reported to have low sensitivity, with a reported estimate of false-negatives [5, 6]. The Possible sources of error include variability among sample takers, cell collection techniques employed, inadequate screening and errors in interpretation [7]. The other causes of false-negative Pap tests include incorrect sampling, absence of atypical cells due to poor fixation and/or overlapping, obscuring haemorrhage and inflammation [7, 8, 9].

In order to improve the sensitivity, specificity, reproducibility and improvements in sample quality there has been a shift from conventional cytology to Liquid based cytology (LBC) with major advances in LBC in the last 15 years [10]. ThinPrep and SurePath are the two Food and Drug Administration (FDA) approved liquid-based preparations used to obtain such smears [11]. The technique of LBC allows the cells to be suspended in a monolayer (without any obscuring blood, inflammation and overlapping of cells), allowing better morphological assessment [12].

LBC allows for additional testing such as human papillomavirus (HPV) DNA testing, other ancillary techniques like immunocytochemistry and cell blocks which can be performed on the residual sample, along with an added advantage of increased detection of precancerous lesions when compared to conventional smears [13]. However, a disadvantage of the LBC system is the high-cost automated processing equipment [14].On the other hand, Manual Liquid-Based Cytology (MLBC) is a technique that enables cells to be suspended in a monolayer similar to LBC and improves detection of precursor lesions and improvement of specimen adequacy [8]. MLBC is an alternative more cost effective strategy in limited resource setting [8].

In our present study we are endeavouring to study Manual method of liquid-based cytology versus Conventional Pap smear.

MATERIALS AND METHODS

Prospective comparative study of 210 cervico-vaginal smear samples collected from patients who attended the Gynecology outpatient clinic at a tertiary care center for 2 years. Patients presenting with history of white discharge per vagina, bleeding per vagina, post coital bleeding, irregular menstrual bleeding and for routine cervical cancer screening, were selected for the study after obtaining an informed, valid, written consent from patients. Institutional Ethical Committee Clearance was obtained for the study.

Pregnant females, age less than 20 years and diagnosed cases of cervical cancer were excluded from the study.

Sample Collection/Processing-

Plastic Ayre's spatula and endocervical brush were used to obtain the sample. Spatula was rotated against the ecto-cervix for a 360^0 to include the transformation zone. Split sample method was followed where the material on one side of the spatula and brush was smeared onto glass slides for conventional smear preparation and fixed using cyto-spray. The remaining material from the spatula was rinsed into a bottle containing liquid fixative prepared in our laboratory for MLBC. Liquid fixative was prepared using 0.5 gm of sodium chloride, 0.5 gm of sodium citrate, 50 ml of 10% formalin and 50 ml of isopropyl alcohol and glacial acetic acid in 100ml of solution. The processing of slides were done as follows after 20-24 hours of fixation

1. Specimen sample was centrifuged at 2000 rpm for 5 minute
2. Supernatant was decanted and excess fixative was blotted
3. 1-2 ml of polymer solution added to the sediment
4. Centrifuged at 1500 rpm for 10 min
5. A direct smear was made taking the sediment with the help of a pipette onto a clean glass slide in circular movements which was fixed with cyto-spray and dried overnight.

Conventional and MLBC smears were both stained with Papanicolaou stain after fixing in alcohol for 2 hours. The smears were reported according to the Bethesda system for reporting cervical cytology. Sensitivity, specificity, positive predictive value and negative predictive value was calculated for which histopathological examination was done, considering histopathological examination as the gold standard. Differences between conventional Pap smears and liquid-based cytology were analyzed and associations of cytological subtype by Bethesda system of classification were tested by kappa statistics. A p value of <0.05 was taken as significant.

RESULTS

In our study we observed the age of the patient's age ranged from 21 to 70 years, with a mean age of 43.75 ± 12.12.

The most common clinical presentation was white discharge per

vagina (72 cases) followed by dysfunctional uterine bleeding (58 cases) and Post-menopausal bleed (34 cases) Of the 210 cases in our study, the satisfactory smears obtained by the method of conventional Pap smear were 196 cases (93.33%) and 14 cases (6.66%) were unsatisfactory, and 205 cases (97.61%) using Manual Liquid-based cytology were satisfactory and 05 cases (2.38%) were unsatisfactory (Table 1)

Table 1: Adequacy of samples using CPS and MLBC

SMEARS	CPS	PERCENTAGE	MLBC	PERCENTAGE
SATISFACTORY	196	93.33	205	97.61
UNSATISFACTORY	14	6.66	05	2.38
TOTAL	210	100	210	100

The other morphological parameters used to compare MLBC and CPS are as shown in Table 2. All the smears were reported according to The Bethesda System of reporting cervical cytology (Table 3). Majority of cases were NILM with inflammation at 52.85% and 51.42 % for MLBC and CPS respectively

Table 2: Morphological parameters to compare CPS and MLBC (210 cases)

MORPHOLOGICAL FEATURES		CPS	MLBC
Cellularity	Adequate	196	205
	Inadequate	14	05
Cleanliness of background	Obscuring factors noted	Present in most of the cases	
Uniformity of distribution	Absent	Present in most	
Artifacts	Rare	Present in few/rare	
Celullar Overlapping	Marked	Mild	
Architectural changes	Present	Absent	
Nuclear changes	Clear	Clear/better observed	

Table 3: Distribution of cases according to the Bethesda System on conventional Pap smear

The Bethesda System Categories	Frequency		Percentage	
			CPS	MLBC
	CPS	MLBC		
Unsatisfactory	14	05	6.66%	2.38%
NILM without inflammation	20	20	9.52%	9.52%
NILM with inflammation	108	111	51.42%	52.85%
Atrophy	04	04	1.90%	1.90%
Trichomonas vaginalis	07	09	3.33%	4.28%
Candida	14	14	6.66%	6.66%
Bacterial vaginosis	00	00	-	-
Actinomyces sp.	00	00	-	-
Herpes simplex virus	00	00	-	-
Cytomegalovirus	00	00	-	-
ASC-US	03	01	1.42%	0.47%
ASC-H	02	00	0.95%	-
LSIL	10	13	4.7%	6.19%
HSIL	10	12	4.7%	5.71%
SCC	16	19	7.61%	9.04%
Atypical glandular cells	00	00	-	-
Adenocarcinoma	02	02	0.95%	0.95%
Other malignant neoplasms	00	00	-	-
Total	21 0	210	100.00%	100.00%

Histopathological diagnosis based morphology was obtained in 50 cases, and were categorized as chronic cervicitis with squamous metaplasia, (26.00%) Cervical intraepithelial neoplasia I (CIN I) (18.00%), CIN II(8 .00%), CIN III (12.00%) , Squamous Cell Carcinoma (32%), non-keratinising squamous cell carcinoma and endometriod adenocarcinoma (2.00%)

In MLBC the histopathological diagnosis correlated with 49 cases and 1 case was discordant. In CPS the diagnosis correlated with 44 cases and 6 cases were discordant (Table 4). We identified 12 cases as chronic cervicitis with squamous metaplasia and with MLBC 11 cases correlated with biopsy findings. Category of CIN I had 10 cases on histopathology and all cases correlated with MLBC, while 06 cases correlated with CPS findings.

Table 4: Histopathological correlation seen on CPS and MLBC smears.

HISTOPATHOLOGICAL CORRELATION	CPS	MLBC
YES	44(88%)	49(98%)
NO	06(12.0%)	01(2.0%)
TOTAL	50	50

In the category of CIN II and CIN III total of 10 cases were obtained, of which all cases correlated with MLBC smears and using CPS 05 cases correlated with biopsy findings.

A total of 17 cases of squamous cell carcinoma (keratinising and non-keratinising) were diagnosed on biopsy and all correlated with MLBC smears. While 15 cases correlated using CPS.

Manual liquid-based (MLBC) method was found to have a higher sensitivity (100%) and specificity (91.7%) in diagnosing lesions than conventional smears which had sensitivity (75%) and specificity (85.7%).The diagnostic accuracy of MLBC was 98% and that of Conventional method was 78%. The p value was less than 0.005 in MLBC (0.0001) and CPS (0.0001) methods.

DISCUSSION

Sampling is the corner stone for cytology and especially true for cervical cytology, Manual Liquid-Based Cytology (MLBC) could be the most appropriate choice to reduce sampling errors and presumably transfer all the cellular material avoiding heterogeneity problems [1, 8, 15]. In our study of MLBC, the samples were collected and processed in a similar way to studies done by Nandini NM et al, and Maksem et al [8, 16]. Age group in our study varied from 20 to 70 year, which was similar to the studies done by Bukhari et al and Jena et al[17, 18,].The most common clinical presentation in our study was white discharge per vagina similar to the studies done by Verma et al and Bal et al[17, 19, 20].

The number of satisfactory smears were increased by method of MLBC (97.61%) compared to CPS (93.33%) which was concordant with studies done by Nandini et al which showed a result of 99% for MLBC and 91% for CPS, Sherwani et al also showed similar results [8, 21]. The better smears on MLBC was due to the method of specimen collection in a preservative solution and rinsing of the sampling device in a liquid fixture which helped the entire sample to be captured into the vial, hence the cellular structure was better preserved as the cells were immediately fixed. All drying artefact and cytolysis was almost absent or minimal using MLBC because of immersion of cells into liquid fixative and specimen adequacy was greatly improved wihtout limiting factors like contaminating mucus, red blood cells, bacteria, yeast and inflammatory cells [11, 21]. (Figure 1a, 1b)

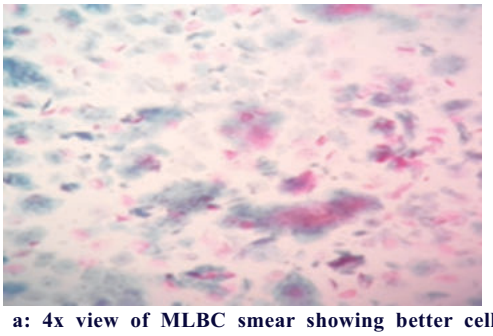

Fig 1 a: 4x view of MLBC smear showing better cellular morphology and no obscuring factors.

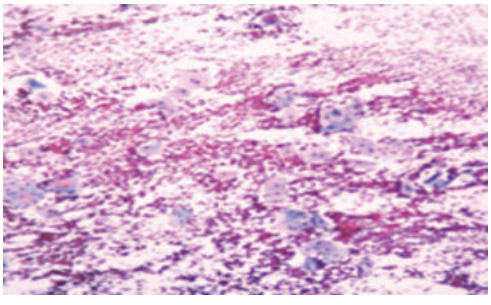

Fig 1b: 4x view of conventional Pap smear, morphology of cells obscured by hemorrhage

Most of the unsatisfactory smears on CPS (6.66%) were due to scant cellularity, presence of blood and mucus, too thick a smear with inflammation obscuring cellular details [1, 8, 22]. The unsatisfactory smears of MLBC (2.38%) haziness obscuring the cellular details (cells entrapped into the polymer membrane of the preservative solution due to inadequate rinsing), and presence of folded cell borders which was similar to the studies done by Moosa NY et al and Bollmann et al[1, 9].

Both methods were efficient in detecting infectious agents, we obtained a result of 10% using CPS and 10.95% using MLBC .Similar findings were reported by Nandini NM et al in which CPS showed 9% and MLBC showed 15% [8]. The organisms were better observed on MLBC than CPS because of the cleanliness of background and reduced number of inflammatory cells. (Figure 2 a, 2b)

The number of cases diagnosed as ASC-US in our study was 1.42% on CPS and 0.47% on MLBC similar to the studies by Khaniki et al The ambiguity of diagnosing cases as ASC-US was decreased on MLBC owing to better morphology and reduced architectural changes of the cells [7, 22]. In the category of ASC-H no case were diagnosed using MLBC and 0.95% cases were diagnosed on CPS.

Increased detection of cellular abnormalities by LBC method depends on many factors like adequacy of sample, and type of sampling like whether direct to vial or split sample method was followed. The main limitations of split-sample method which we followed in our study to compare MLBC and CPS had a decreased rate of endocervical cells on MLBC, as the procedure was carried out on the residual sample on the spatula and cervibrush, which is also discussed in studies by Rosenthal DL et al and Stabile et al [23, 24]. This limitation of split-sample method can be overcome by direct sampling method or collecting the sample for LBC in liquid medium [15].

In the category of LSIL, MLBC detected 6.19% compared to CPS which detected 4.7%. (Figure 2 a,b). MLBC detected 5.71% in the HSIL category while CPS detected 4.7% (Figure 3). Similar observation was shown in study done by Sherwani et al in which MLBC detected 18.1% and CPS detected 36%. However there was not much difference in detection of HSIL by two methods [21]. The cases of Squamous cell carcinoma (keratinising and non-keratinising) (Figure 4) diagnosed in our study using MLBC was 9.04% compared to CPS which was 7.61%. Many studies concluded that in diagnosing malignancies CPS was comparable to LBC and not much of statistically significant difference was noted [8, 19, 21].

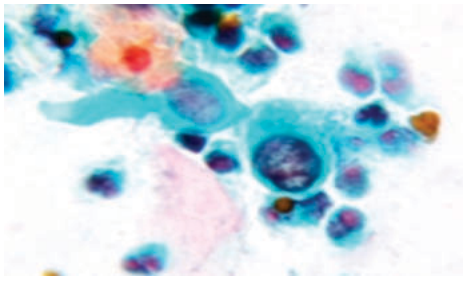

Fig 3: 40x view of MLBC smear showing HSIL

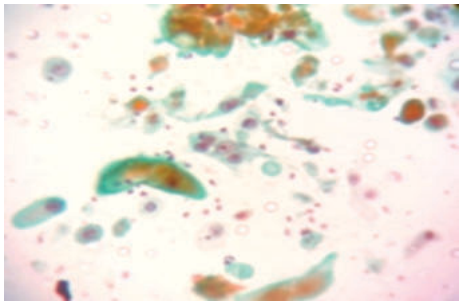

Fig 4: 40x view of MLBC smear showing squamous cell carcinoma

Under the category of CIN I, II, and III MLBC had 100% correlation to biopsy findings whereas CPS had correlation of 60% for CIN I, the cases which did not correlate on biopsy were diagnosed as inflammatory and HSIL on smears. In diagnosing CIN II, and CIN III CPS had 50% correlation, discordant cases were diagnosed as inflammatory and LSIL on smear. In diagnosing malignancies MLBC had 100% correlation compared to CPS which had 88.23%, discordant cases were diagnosed as HSIL on smears because of drying artefact, which was similar to study done by Sherwani et al [21].

The sensitivity of MLBC was 100% and specificity was 91.7% while CPS had sensitivity of 75% and specificity of 75.7% which was similar to the studies [Table 5]. A single case reported as ASC-US on MLBC in our study was diagnosed as inflammation on biopsy probably due to the better cellular details and nuclear enlargement seen on smear, which was similar to study done by Kavatkar et al [11].

The diagnostic accuracy of MLBC was 98% and CPS was 78%, which was similar to study by Kavatkar et al in which they found a higher diagnostic accuracy and significantly lower error rates using method of MLBC than CPS [11].

Table 5: Comparision of rates of Sensitivity and Specificity of various study and present study

Authors	Sensitivity (%)		Specificity (%)	
	LBC	CPS	LBC	CPS
Abulafia et al (2003)	76	68	86	79
Sherwani et al (2007)	97.6	53.7	50	50
Behtash et al (2008)	83	66	98	86
Khaniki M et al (2008)	83	66	98	33
Nandini NM et al (2012)	100	75	100	50
Verma K et al (2014)	80	52	99	98
Present Study	100	75	91.7	85.7

CONCLUSION

MLBC method has advantages over CPS in diagnosing cases because of the well fixed samples leading to better morphology of cells, better uniformity in distribution, and absence of obscuring factors like blood and inflammation with an addition of shorter screening time.

Also the sample collected for MLBC can be used for immunocytochemistry and HPV DNA testing if required. In developing countries, MLBC is a cost effective alternative to the expensive automated LBC equipment. MLBC is a comparable effective test to CPS, keeping in mind the time taken for sample processing, it can be considered as an adjunctive to CPS, especially in doubtful cases with the need of better cellular and nuclear morphological assessment, leading to precision in diagnoses and triaging patients accordingly for treatment with reduction in the burden of morbidity and mortality due to cervical cancer.

Acknowledgment

The authors confirmed contribution to the article as follows: Study design; Dr Shwetha. Laboratory analysis and support; Mr Abdul Raheem and Mr Nagaraj. Data collection; Dr Hema, Data analysis and interpretation; Dr Mahesh Hulgeri, Review and editing; Mr Omer

Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Declaration of conflicting interests

There are no conflicting interests

REFERENCES

1. Moosa NY, Khattak N, Alam MI, Sher A, Shah W, Mobashar S, et al. Comparison of Cervical Cell Morphology Using Two Different Cytology Techniques for Early Detection of Pre-cancerous Lesions. Asian Pac J Cancer Prev 2014; 15(2):975-81.
2. Kalyani R. Virus and Cervical Cancer: Role and implication: A review. Biomedical Research and Therapy 2015; 2(3):220-30.
3. Basu P, Chowdhury D. Cervical cancer screening & HPV vaccination: a comprehensive approach to cervical cancer control. Indian J Med Res 2009; 130:241-46.
4. Sreedevi A, Javed R, Dinesh A. Epidemology of cervical cancer with special focus on India. Int J Womens Health 2015; 7:405-14.
5. Cibas ES, Ducatman BS. Cytology: Diagnostic Principles and Clinical Correlates.4th ed. China: Saunders Elsevier; 2014.p.2-11.
6. Lopez-Cuervo JE, Beltran EM, Lopez JLC, Castillo AA, Sanchez TN. Preliminary Study of a New, Fully Automated System for Liquid-Based Cytology: The NovaPrep Processor System. ActaCytol 2011; 55:281-6.
7. Behtash N, Nazari Z, Khaniki M, Zendedel K, Fakor F, Shariat M. LiquiPrepTM a new liquid-based cervical cytology method in comparison with conventional Pap smear in developing countries. Res J BiolSci 2008; 3(6): 627-30.
8. Nandini NM, Nandish SM, Pallavi P, Akshatha SK, Chandrashekhar AP, Anjali S, et al. Manual Liquid-based Cytology in Primary Screening for Cervical Cancer- a Cost Effective Preposition for Scarce Resource Settings. Asian Pac J Cancer Prev 2012; 13:3645-51.
9. Bollmann R. Liquid-based cytology. Gynakol Geburtsmed Gynakol Endokrinol 2008; 4: 164-80.
10. Gibb RK, Martens MG. The Impact of Liquid-Based Cytology in Decreasing the Incidence of Cervical Cancer. Rev Obstet Gynecol. 2011; 4(1): S2–S11.

11. Kavatkar AN, Nagwanshi CA, Dabak SM. Study of a manual method of liquid based cervical cytology. Indian J Pathol Microbiol 2008; 51:190–4.
12. Pawar PS, Gadkari RU, Swami SY, Joshi AR. Comparative study of manual liquid-based cytology (MLBC) technique and direct smear technique (conventional) on fine-needle cytology/fine-needle aspiration cytology samples. J Cytol 2014; 31(2):83-86.
13. Cutts FT, Franceschi S, Goldie S, et al. Human papillomavirus and HPC vaccines: a review. Bull World Health Organ 2007; 85(9):719-26.
14. Rahangdale L, Budwit D, Asgari D, Ohadugha AL. Manual Liquid-Based Cytology: A Clinical Pilot Study of the VitroPrep™ Cytology Processing Kit. ActaCytol 2014; 58:373-77.
15. Bigras G, Rieder MA, Lambercy J, Kunz B, Chatelain JP, Reymond O et al. Keeping Collecting Device in Liquid Medium Is Mandatory to Ensure Optimized Liquid-Based Cervical Cytologic Sampling. Journal of Lower Genital Tract Disease 2003; 7(3):168–174.
16. Maksem JA, Dhanwada V, Trueblood JE, Weidmann J, Kane B, Bolick DR, et al.Testing automated liquid-based cytology samples with a manual liquid-based cytology method using residual cell suspensions from 500 ThinPrep cases. DiagnCytopathol 2006; 34:391-96.
17. Bukhari MH, Saba K, Qamar S, Majeed MM, Niazi S, Naeem S. Clinicopathological importance of Papanicolaou smears for the diagnosis of premalignant and malignant lesions of the cerivix. J cytol 2012; 29(1): 20-47.
18. Jena A, Bharathi T, Reddy YKS, Manilal B, Patnayak R, Phaneendra BV.Papanicolaou test screening of staff members in a tertiary care teaching hospital in South India. J ClinSci Res 2012; 1:174-7.
19. Verma K. Clinical assessment and correlation of pap smear and liquid-based cytology in bad cervix. Journal of Evolution of Medical and Dental Sciences 2014; 3(53): 12277-87.
20. Bal MS, Goyal R, Suri AK, Mohi MK. Detection of abnormal cervical cytologyPapanicolaou smears. J Cytol 2012; 29(1):45-7.
21. Sherwani RK, Khan T, Akhtar K, Zeba A, Siddiqui FA, Rahman K et al. Conventional Pap smear and liquid-based cytology for cervical cancer screening- a comparative study. J Cytol 2007; 24(4):167-172.
22. Khaniki M, Nazary Z, Zendehdel K, Fakur F. Cervicovaginal Cytopathology by Liquiprep™ a New Liquid Based Method in Comparison with Conventional Pap Smear. Iran J Pathol 2009; 4(2):59-64.
23. Rosenthal DL, Geddes S, Trimble CL, Carson KA, Alli PM. The PapSpin: A Reasonable Alternative to Other, More Expensive Liquid-Based Papanicolaou Tests. Cancer Cytopathol 2006; 108:137-43.
24. Stabile SA, Evangelista R, Talamonte VH, Lippi UG, Lopes RG. Comparative study of the results from conventional cervico-vaginal oncotic cytology and liquid-based cytology. Einstein Q J Biol Med 2012; 10(4):466-72.