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Comparison between manual liquid based cytology and conventional Pap smear for evaluation of cervical lesions and it's histopathological correlation in cases of epithelial abnormalities

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Abstract

Introduction: Pap smear has been most widely used screening method for cervical cytology since last 50 years. Despite of it reducing morbidity and mortality from cervical cancer by detecting precancerous lesions, false negativity rate of Pap smear is very high. Manual liquid based cytology has been developed as an alternative to conventional Pap smear to overcome its drawbacks. Main advantages of MLBC over CPS are increased percentage of representative cells, better presentation on slide, increased detection of abnormal or dysplastic cell and overall reduced number of unsatisfactory smears.

Aim: to compare the number satisfactory smears and sensitivity in detecting intraepithelial abnormalities with both the techniques.

Materials and Methods: this was a prospective study done on 200 symptomatic women. The sample of each patient was collected into a preservative vial by a cytobrush for MLBC and with an Ayre spatula directly on a slide for CPS. The results were interpreted according to the Bethesda system. The cases of intraepithelial lesions on cytology were followed and confirmed by histopathology.

Results: *MLBC* showed slightly higher number of satisfactory smears ie. 175 (87.5 %) as compared to CPS (171 ie. 85.5%). The sensitivity of MLBC was much higher ie. 81.8% as compared to CPS which was 36.3%. The p- value was calculated as 0.15 which was not significant.

Conclusion: *MLBS* was better than CPS in specimen adequacy and lesser obscurance of morphology of representative cells. The number of intraepithelial lesions detected on MLBC was higher but its superiority over CPS is still debatable.

Keywords: cervical cytology, Manual liquid based cytology, conventional Pap smear, intraepithelial lesions.

Introduction

Cervical cancer is the second most commonly diagnosed cancer worldwide and third leading cause of cancer death among females in less developed countries.^{1, 2, 3}

Most common type of cervical cancer is squamous cell carcinoma arising from the squamous epithelial lining of cervix.⁴ Squamous intraepithelial lesion can be detected earlier before it transforms into invasive cancer which is the basis of cervical cytology screening. P1 Main

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cause of this transformation is Human Papilloma Virus (HPV) ⁵ In 1928, George Papanicolaou discovered that exfoliated cells from a growth in cervix can be seen in a vaginal smear.^{6,7}

Wide use of Pap smear as a cancer screening tool for the cervix at an early stage has been substantiated by several studies to decrease in incidence and mortality of cervical cancer.⁸

Conventional pap smear yields high number of false negative results, main drawback being over 90% of the cells are discarded along with the spatula and the proportion of cellular material which is transferred may include all, none or just some of any abnormal cells. Also irregularity in the thickness of smears leads to obscurence of cell morphology by blood and mucus.⁹

Liquid Based Cytology (LBC) was introduced in mid 1990s to overcome these disadvantages of the conventional Pap test.¹⁰

It is processed in such a way that a thin layer (monolayer) of cervical epithelial cells are deposited in a diameter of 13 mm. ⁹ Main advantages of LBC are higher percentage of representative cells obtained. Since it is a monolayer preparation, there will not be any overlapping of cells. Mucous and blood are removed during the processing and hence the cellular morphology is clearly seen. ⁹ The present study was undertaken to compare the morphology of various cervical lesions on manual LBC and CPS and sensitivity of both the techniques.

Materials and Methods

200 women over 25 years of age attending Gynaecology OPD were selected presenting with the complaints of irregular menstrual bleeding, abnormal vaginal discharge, pain in lower abdominal, post-menopausal bleeding, post coital bleeding or growth or ulceration in the cervix. A detailed history was taken. All selected women were examined per vaginally and by speculum after acquiring a detailed history and verbal consent from them. The woman was placed in dorsal lithotomy position. After proper positioning of the woman, cervix was viewed by introducing Sims' vaginal speculum and anterior vaginal retractor and external os was identified. Pap smears were made by introducing Ayer spatula and rotating it through 360 degrees near the squamo- columnar junction. The cellular material thus obtained was quickly, but gently smeared on a clean glass slide. The glass slide was then immediately put into the Coplin jar containing 95% ethanol which acted as a fixative. The prepared smears were then stained according to Papanicolaou's technique. Liquid based cytology smears preparing by using cervical brush/ cytobrush with a detachable head were inserted into the external os and rotated 8-10 times in clockwise direction. The white head of the cervical brush was detached and put into the Sure Path preservative vial . Vial was then shaken well and stored at room temperature till the samples were processed for cytology.

For processing of manual liquid based sample these steps were followed-

- 1. Vortexing
- 2. Repeated centrifugation at different rpm and discarding of the supernantant to obtain a cell button which is resuspended.
- 3. Fixing and staining of sample with pap satin in settling chambers.

Both conventional pap smears and smears prepared by MLBC were interpreted as per the Bethesda system of reporting of Pap smears.

Results

Most of the symptomatic patients belonged to the age group 36-45 years (36.5 %) and minimum above the age of 65 years. Most commonly presented symptom was abnormal discharge per vaginum. Among 200 conventional smears, 171 (85.5 %) were satisfactory and 29 (14.5 %) unsatisfactory of which mostly were due to clumping and overlapping rest was related to obscurence with inflammatory cells, blood and mucus. MLBC showed 175 (87.5 %) smears as satisfactory. However, MLBC showed a little difference in

satisfactoriness of smears, no unsatisfactory smear on MLBC was found to be due to clumping or overlapping of the cells.107 (53.5%) smears were inflammatory by MLBC method and 127 (63.5%) were inflammatory by CPS method.

Detection of Trichomonas vaginalis turned out to be same that is 01(0.5%) by both the techniques. Findings of Bacterial vaginosis was seen in 06 cases (3%) by MLBC while 03 cases (1.5%) by CPS.(Table 1) Detection of intraepithelial lesions was more on MLBC as it detected 24 intraepithelial lesions (ASCUS -06; LSIL- 03; HSIL – 15). CPS detected 12 intraepithelial lesions (ASCUS -04; LSIL- 02; HSIL – 06). (Table 2, Figure 1)

Result	LB	LBC		CPS	
	Number	%	Number	%	
Normal	30	15	28	14	
Inflammation	107	53.5	127	63.5	
Bacterial Vaginosis	06	3.0	03	1.5	
Trichomonas Vaginalis	01	0.5	01	0.5	
ASCUS	06	3.0	04	2.0	
LSIL	03	1.5	02	1.0	
HSIL	15	7.5	06	3.0	
Unsatisfactory	25	12.5	29	14.5	
Total	200	100	200	100	

Table 1: Frequency of Cervical Lesions Detected on MLBC and CPS

Table 2 Comparison between Cytological Interpretation by MLBC and CPS

Result	MLBC (%)	CPS (%)
Normal	37 (18.5%)	28 (14.0%)
Inflammation	114 (57.0%)	132 (66.0%)
Epithelial Cell Abnormality	24 (12.0%)	12 (6.0%)
Unsatisfactory	25 (12.5%)	29 (14.5%)
Total	200 (100%)	200 (100%)

Figure 2 Comparisons between MLBC and CPS in detecting various Cervical Abnormalities



22 cases out of total 29 cases positive for epithelial abnormality were followed and confirmed by histopathological examination of cervical biopsy. Conventional Pap smear could detect 12 out of 29 cases positive intraepithelial lesions. 8 positive cases were confirmed by

histopathology ie. True positive. Therefore sensitivity of CPS is 36.3%. Liquid based method could detect 24 intraepithelial lesions out of which 18 were confirmed positive by histopathology ie. True positive. Therefore sensitivity of MLBC is 81.8%. (Table 3)

Table 3Histopathological and CytologicalCorrelation

	LBC	CPS
Normal/Inflammatory	4	14
(False Negetive)		
Abnormal (ASCUS, LSIL, HSIL)	18	8
(True Positive)		
Total Positive Cases Confirmed	22	22
On Histopathology		

Discussion

The Pap smear has been utilized for cervical cancer screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two third of the overall false negative rate can be attributed to sampling errors.¹¹ Liquid based cytology has been developed to address the sampling problems of conventional Pap smear. In our study, 200 women were screened and their interpretation by both methods was compared. Most of the symptomatic patients who were screened belonged to the age group 36-45 years (36.5%) followed by 25-35 years (35.5%) which was similar to Sherwani et al ¹² who studied 160 random cases out of which 48.1% cases belonged to fourth decade of life. Positive cases in which epithelial abnormalities were seen such as ASCUS, LSIL and HSIL were found to be positive mostly between age group 46- 55 years i.e. 12 patients (42.8 %) similar to other studies like that of Terence Colgan et al.¹³ Most common symptom, was irregular bleeding in 57.5 % cases (115 cases), followed by pain lower abdomen in 54.5 % cases (109 cases) and post menopausal bleeding seen in 15 % cases (30 cases) and growth or ulceration over cervix seen in 5 % cases (10 cases). This was discordant with most of the other studies which observed white discharge per vaginum as the complaint in most of the patients ^{12,14,15} that may be due to the fact that we selected symptomatic patients only and females with no symptoms or not seeming to be in a high risk zone were excluded from the study. Most of the cases 67.8 %) positive for (19 ie. epithelial abnormalities either on MLBC or CPS came with the complaint of post menopausal bleeding. Mostly (58.5 %) cases belonged to parity less than Para 4. Parity was found to be in direct relationship with intraepithelial lesion as 21 out of 29 positive cases ie. 72.4% cases had more than 3 children (para 4 or more). This was cordant with the study of Vibhuti Garg et al ¹⁴ and Khushboo who epithelial Verma et al^{15} founded abnormalities mostly in multipara women. In our study 200 conventional smears, (85.5 %) were satisfactory and 29 (14.5 %) unsatisfactory. Among 200 liquid based smears, 175 (87.5 %) were satisfactory and 25 (12.5%) unsatisfactory. Pearson chi square value for comparison between satisfactory smears on MLBC and CPS was calculated as 2.00 but not significant (p- value <0.15).Our study was similar to Dhanajay et al ⁴ in which there was increase in the number of satisfactory smears on MLBC (88.7%) as compared to CPS (86.6%). Also study by Joel et al 16 , the conventional test had 4.4% (23/525) unsatisfactory results, vs. only 1.7% (9/525) unsatisfactory results from the liquid based test. However, in a few studies, increase in number of satisfactory smears on LBC was significant like Singh et al ²⁰ observed 92.5% satisfactory smears on LBC while 78.8% on CPS. (P value = 0.02). Erdin and Ahmet et al ²¹ observed lesser number of unsatisfactory smears on LBC (0.05%) than the CPS method (0.5%) (P < 0.001). Most of the unsatisfactory smears in our study on conventional method were due to clumping and overlapping rest was related to obscurence with inflammatory cells, blood and mucus. In our study out of 200 cases, 103 (52.5%) smears were

inflammatory by MLBC method and 128 (64%)

were inflammatory by CPS method. Out of 24

smears (12.0%) with features of epithelial

abnormalities (ASCUS, LSIL, HSIL) on MLBC, 6

(3%) were ASCUS, 3(1.5%) were LSIL and 15(7.5%) were HSIL. CPS could detect 12(6.0%) smears with features of epithelial abnormalities (ASCUS, LSIL, HSIL) out of which 4(2.0%) were ASCUS, 2(0.5%) were LSIL and 6(3%) were HSIL. In our study all the epithelial lesions were detected more by MLBC method. Number of ASCUS detected on MLBC was 1.5 % higher, LSIL were 1% higher and HSIL were 3.5 % higher than CPS. Pearson chi square value when the positive results for detection of epithelial abnormality was 16.206 and p-value= 0.06. (not significant) This was in cordance with study of Terence J Colgan¹³ which also showed an increasing trend in detection of ASCUS by 0.88%, LSIL by 0.63% and HSIL by 0.03% on LBC over CPS but this trend could not reach the significant limit (p-value=0.0696). Another study bv Khushboo Varma et al ¹⁵ also found a slightly higher rate of detection of ASCUS by 2.94% and HSIL by 11.76% with LBC method than CPS method but still not significant. Joonseok Park et al ²²also observed increasing trend in detection of ASCUS by 3.9%, LSIL by 4.3% and HSIL by 1.9%. Badri Lal Patidar et al ¹⁹ also correlated their cytological findings with histopathology and observed that out of total 16 cases positive on biopsy, 14 cases were positive on LBC, so sensitivity of liquid based cytology was 87.50%. Other studies also showed an increase in sensitivity by LBC as compared to CPS like:

	Sensitivity	
	LBC	CPS
Singh & Gupta et al ¹⁷	92.5%	78.8%
Chinaka et al ⁵	100%	86.0%
Nandinin NM et al ²³	75%	50%
Deshou et al ¹⁰	95.4%	78.9%
Sherwani et al ¹²	97.6%	53.7%
Present study	81.8%	36.3%

Conclusion

Manual liquid based cytology introduced as an alternate technique to some extent produces a monolayer smears which are easier for interpretation as there is no overlapping or clumping and cells with atypia are not obscured by other of cells or background (inflammation, blood, mucus etc). However, the superiority of LBC over CPS is debatable. From our study, it was inferred that smears prepared by MLBC technique showed clearer background, well preserved cytomorphological details, removal of excess mucus, blood and inflammatory cell infiltrate as compared to CPS technique. Atypical cells or abnormal cells were better seen by MLBC as compared to CPS. On comparison of both techniques, epithelial abnormalities such as ASCUS, LSIL and HSIL were detected more easily and frequently by MLBC method as compared to CPS technique. In our study the sensitivity of MLBC in detecting epithelial abnormalities is much more than CPS. However, p-value was not significant indicating that both the methods are comparable and neither is superior to the other.

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