

## Conventional pap (papanicolaou) smear cytology in primary screening of cervical lesions & its comparison with manual liquid based cytology

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### Abstract

**Background:** Conventional pap smear (CPS) examination has been the mainstay for early detection of cervical cancer. However, its widespread use has not been possible due to the inherent limitations, like presence of obscuring blood and inflammation, reducing its sensitivity considerably.

**Aims & Objectives:** The study was performed to compare the efficacy of Manual Liquid based Cytology (MLBC) with CPS and to find out whether MLBC can be established for routine use in our laboratory setup.

**Materials & Methods:** Cervical smears were collected by the gynaecologist using Ayre's spatula and cytobrush for the conventional Pap smear method and are suspended in a liquid medium for manual liquid based cytology technique. In this study, cervical screening was performed in 481 cases, all were married women of reproductive age group who visited Department of Obstetrics and Gynaecology over a period of one year from July 2014 to June 2015. Slides were examined for cytomorphological parameters. The details regarding cell size, cytoplasmic and nuclear details were studied for making the diagnosis.

**Result:** The sensitivity of CPS in detecting cervical lesions is 95% and by MLBC is 50% while the specificity was 86% with CPS technique and 69% by MLBC technique.

**Conclusion:** The cellular features are better in MLBC as compared to CPS and also background is more clear in MLBC as there was no obscuration by RBCs or inflammatory cells while in CPS, the background is dirty due to the presence of RBCs, necrosis and inflammation.

**Keywords:** Conventional Pap Smear, Manual Liquid based Cytology, Pap smear, Cervical cytology.

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### Introduction

Cervical cancer is leading cause of mortality and morbidity among women worldwide and is the third most common cancer in the world.<sup>1</sup>

The cervical smear was pioneered by Dr George Papanicolaou starting in 1920s. Credit for decrease in cervical cancer death should go to the Papanicolaou cytologic test or the Pap smear as we know it today.<sup>2</sup>

Smears prepared by MLBC can be read more quickly than CPS slide, other advantage includes improved sensitivity & specificity, since fixation is better with clear background & nuclear details are well preserved.<sup>3</sup>

The study was performed to compare the efficacy of Manual Liquid based Cytology (MLBC) with conventional pap smear(CPS).

### Materials & Methods

The study included 481 patients in the reproductive age group of 21-45 years. They were registered as OPD patients in the Department of OBG with complaints of discharge per vaginum, pain lower abdomen and back, menstrual irregularities and post coital bleeding. Pregnant females and females less than 20 years of age were excluded from the study. After taking a detailed history and doing thorough clinical examination, Pap smear was taken. It was ensured that patient was not on any intravaginal drug and abstained from coitus a day prior to examination.

A plastic Ayre's spatula was used to collect the samples. Spatula was rotated against the ectocervix for a full rotation so as to include the transformation zone components. Split sample method was followed wherein material from one side of the spatula was spread onto a clean glass slide and fixed in 95% ethanol for conventional method. The spatula will then dipped into a bottle with fixative prepared in our laboratory (20 ml of isopropyl alcohol + 6ml of glacial acetic acid + 74 ml of normal saline) for 10 minutes. The specimens was subjected to two methods for morphological diagnosis namely CPS and MLBC and stained by Papanicolaou staining method.

### Observations

A total of 481 married females in the reproductive age group, attending Department of Obstetrics and

Gynaecology, over a period of one year were screened by Pap smear examination. The results were observed and analyzed.

Maximum number of females 146(30.35%) were in the age group of 21-25 years. Youngest female examined was of 21 years of age and oldest was 45 years of age. (Table 1)

Majority of women in this study were para 3-4 (75.50%) and 13.24% of cases showed parity >4.

Symptomatic cases were maximum (95.50%) while asymptomatic cases that came for screening were very less.

Most common symptom, was discharge per vaginum in 48.40% cases, followed by pain lower abdomen and back in 32.90% cases while least common was post coital bleeding seen in only 3.00% cases.

On per speculum examination, also the most common finding was discharge per vaginum (242 cases). In about 36.47% women, there was no significant finding and cervix appeared healthy.

Maximum number of patients with complaint of discharge per vaginum were in the age group of 21-25 years. Pain lower abdomen and back and menstrual irregularities were most common in 26-30 years of age group whereas post coital bleeding was common in 36-40 years of age.

Maximum number of cases with non-specific inflammation were in the age group of 26-30 years. Candida was seen mostly in 26-30 years of age. Bacterial vaginosis was common in 21-25 years of age while *T. vaginalis* is seen in 36-40 years of age.

Maximum number of epithelial abnormalities were in the age group of 41-45 years.

Most common presenting complaint was discharge per vaginum which is mostly seen in patients with parity 3-4 followed by pain lower abdomen and back and menstrual irregularities.

Maximum number of cases with non-specific inflammation were of parity 3-4. In the specific inflammatory cases, bacterial vaginosis and candida were most common in patients with parity 1-2 whereas trichomonas was seen in patients with parity >4.

Maximum number of patients with epithelial abnormalities were of parity >4.

Of total 481 cases, most common diagnosis on Pap smear examination was inflammatory Pap smear in 416 cases (86.48%) whereas epithelial abnormality was detected in only 10 cases (2.40%).(Table 2)

Of total 416 inflammatory smears, 343 (82.45%) were of non-specific inflammation, while specific inflammation was seen in 73 cases.

Most common organism reported on inflammatory Pap smear was Candida in 34 cases while least common was trichomonas vaginalis in 8 cases.(Table 3)

### Comparison of CPS & MLBC Smears (Fig. 1)

Out of total 481 cases, 346 random cases were selected for comparison by two method- Conventional Pap smear and Liquid based cytology (Manual). Table 3 shows comparative analysis by both techniques.(Fig 1)

Out of total 346 smears, 296(85.78%) smear were inflammatory by CPS and 161(46.1%) smear were inflammatory by MLBC technique. 24 (7%) smear were unsatisfactory by CPS whereas 169 (49%) smears were unsatisfactory by MLBC technique(Table 3). Statistical analysis was performed using chi square test ( $\chi^2$  test) where,  $p < 0.00001$ , i.e. highly significant correlation exists between two methods.

Out of 296 inflammatory cases, 226 cases were found to be of nonspecific inflammatory pathology by CPS method while 70 cases were of specific inflammation (20.70%). However, by MLBC technique, out of 161 inflammatory cases, only 21 cases of specific inflammation were detected along with 140 cases of non-specific inflammation. Statistical analysis was performed using chi square test ( $\chi^2$  test) where,  $p < 0.007$ , i.e. significant correlation exists between two methods.

Candida was the commonest organism found on Pap smear examination by both technique, however its frequency was more in CPS technique as compared to MLBC technique. So, it was observed that overall frequency as well as density of specific inflammatory etiologic agents were much less in MLBC as compared to CPS. However, cytomorphological details were remarkably better in MLBC.

On comparison of both techniques, it was found that epithelial abnormality was detected more by CPS method (10 cases) as compared to MLBC technique (6 cases).(Table 3)

Out of total 346 cases, 322(93%) were satisfactory by CPS method but only 177(51.1%) cases were satisfactory by MLBC method while only 24(6.9%) cases were unsatisfactory by CPS and 169(49%) by MLBC technique.

Table 4 shows cellular features were better in MLBC as compared to CPS. Cellularity was adequate in most cases by CPS technique whereas MLBC showed less number of satisfactory smear while cellular distribution was better with MLBC technique.

Sensitivity of CPS in detecting cervical lesions was 95% and by MLBC was 50% while the specificity was 86% with CPS technique and 69% by MLBC technique.

**Table 1: Age Wise Distribution of Cases (N=481)**

Age group (in years)	Number of cases	% of cases
21-25	146	30.35
26-30	128	26.61
31-35	107	22.24
36-40	56	11.66
41-45	44	9.14
Total	481	100

**Table 2: Results of Cytological Examination by Conventional Pap Smear Test (N=481)**

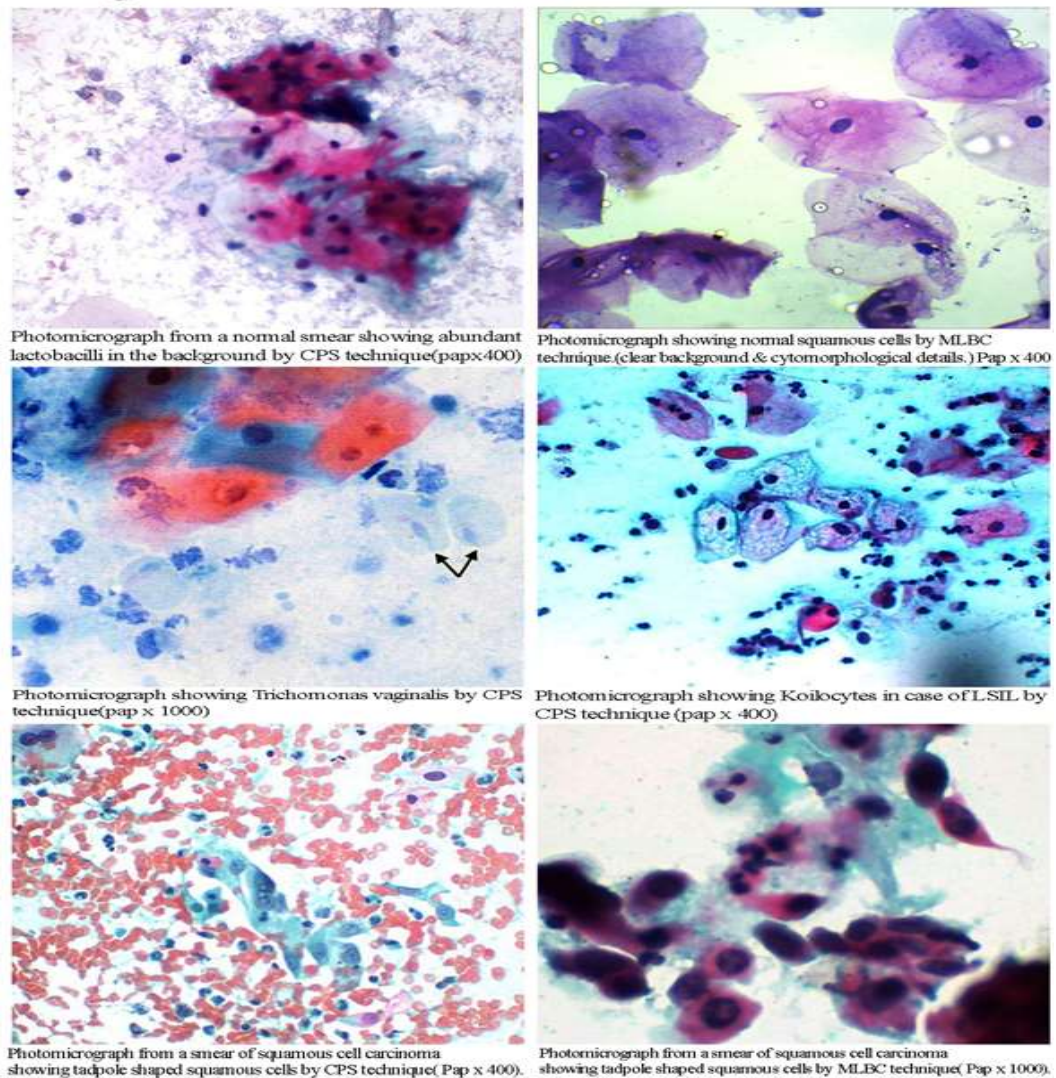
Pap smear findings	Number of patients	% of patients
Normal	29	5.60
Inflammation	416	86.48
Epithelial cell abnormality	10	2.40
Unsatisfactory	26	5.52
Total	481	100

**Table 3: Comparison of CPS and MLBC smears(N=346)**

Smear	CPS	MLBC
Normal	16	10
Inflammation	296	161
1. Candida	38	13
2. B.Vaginosis	27	07
3. T.Vaginalis	05	01
4. Non-specific inflammation	226	140
Epithelial abnormality	10	6
1. Few atypical cells	03	02
2. ASC-US	02	01
3. LSIL	02	01
4. HSIL	01	01
5. SCC	02	01
Unsatisfactory	24	169
Total	346	346

**Table 4: Comparison of cellular features by CPS and MLBC**

Features	CPS	MLBC
Cellular Overlap	Present	Rare
Sheets of cells	Larger	Smaller
Cellularity	Adequate	Inadequate
Cell distribution	Uniform to even	Mostly uniform
Cell size	Larger	Smaller
Cytomorphology	Preserved	Preserved
Background clean	No	Yes
Artifacts	Present	Rare



**Fig. 1: Comparison of CPS & MLBC Smears**

## Discussion

A decline in the incidence and mortality caused by cervical cancer has been observed in the past few decades as a result of screening by cytology. In recent years, the accuracy of the conventional Pap smear has come under a great deal of scrutiny. Investigations into the sources of false negative errors have concluded that the majority are due to sampling error, that is, no abnormal cells were found on the smeared slide upon review as reported by Gay et al.<sup>4</sup> Abnormal cells may also go undetected because of poor smear quality according to Weintraub et al.<sup>5</sup> As a result, liquid based cytology was developed for detection of low-grade intraepithelial lesions, and a significant improvement in specimen adequacy was reported by Bolick et al.<sup>6</sup>

In present study, target group belonged to reproductive age group where age range varied from 21-45 years. Majority of women screened belonged to 21-25 yrs of age. In contrary to present study, Sherwani et al<sup>7</sup> reported 48.1% cases belonged to fourth decade

of life, followed by 31.2% cases in third decade. Study by Takei et al<sup>8</sup> showed age range between 13-79 years and the mean age was 35.6 years. In this study, mean age was 33 years which was very close to that reported by Takei et al.<sup>8</sup>

In the present study, 363 of total women screened were of Para 3-4, finding similar to as reported by Shastri et al.<sup>9</sup> 10 cases were reported as abnormal on Pap smear, of which 6 cases had parity of >4 a finding concordant with the study of Sankarnarayana et al.<sup>10</sup> Parker et al<sup>11</sup> also commented that seven or more parity had a fourfold increase in the risk of developing squamous cell carcinoma of the cervix. Nandani et al<sup>12</sup> also commented that majority of the cases of dysplasia & carcinoma on cervical cytology had parity of five or more.

This study along with various other studies reported discharge per vaginum as the commonest symptom. Kenneth and Yao<sup>13</sup> had emphasized the significance of vaginal discharge and its association

with neoplastic changes in the cervix, they also reported that all patients with post coital bleeding had moderate dysplasia (66.67%) and carcinoma (33.3%).

On per speculum examination most common finding was discharge per vaginum in 50.48% of cases while in about 36.47% of women there was no significant finding and cervix appeared healthy. In only few cases cervix was hypertrophied which was similar to as reported by Sherwani et al<sup>7</sup> in which most common complaint was discharge per vaginum in 42.5% cases followed by pain lower abdomen in 27.5% cases.

In the present study, maximum number of epithelial abnormalities were in the age group of 41-45 years. Sherwani et al<sup>7</sup> reported 77(48.1%) cases in the fourth decade of life, cases of LSIL were mostly found in fourth decade.

In present study, majority of Pap smear examined by conventional technique were reported as inflammatory in 86.48% except in 10 cases where epithelial abnormality was reported. As compared with other studies, Sherwani et al<sup>7</sup> reported 71.87% of cases as inflammatory smears. Luthera et al<sup>14</sup> reported 70% smears as inflammatory while Kawatkar et al<sup>15</sup>, reported inflammatory cases as 66.66%.

In the present study, 10 cases of abnormal Pap smears were reported by CPS technique. Number of abnormal Pap smear got further reduced by MLBC (6 cases) and also density of atypical cell was much less when compared to CPS. Similar findings were reported by Kawatkar et al<sup>15</sup> where 2 cases of HSIL were reported on CPS but were unsatisfactory by MLBC. Various other studies also reported the same. In the present study, reason for less number of cases showing epithelial abnormality could be due to younger age of females screened (<45 years) and as it was a split sample study, majority of cells were used in preparation of CPS slide.

Sherwani et al<sup>7</sup> reported sensitivity 97.6% and specificity as 50% by MLBC and of conventional pap smear 53.7% and 50% respectively. Bolick et al<sup>16</sup> reported sensitivity & specificity of liquid based cytology as 95.2% and 58% whereas on conventional pap smear the same was 85% and 36% respectively. In present study, sensitivity by MLBC was 50% and specificity was 69%.

Manual method of liquid based cytology is an inexpensive, cost effective method of LBC. The other advantages of MLBC method is that the residual specimens can be used for ancillary testing like immunocytochemistry by cell block. Preparation and detection of HPV DNA by PCR or in situ DNA hybridization as stated by Maksem et al<sup>17</sup> and Kavatkar et al<sup>15</sup>.

## Conclusion

From our study, it was inferred that smears prepared by MLBC technique showed clear

background, well preserved cytomorphological details, removal of excess mucus, blood and inflammatory cell infiltrate as compared to CPS technique. However, endocervical cells and cellularity were decreased by MLBC technique could be as it was a split sample study. Atypical cells or abnormal cells were better seen by CPS as compared to MLBC. Reduced number of atypical cells could be due to their relatively fragile nature, thereby destroyed during cytospin processing lab test.

These conclusions may not be totally applicable to automated LBC techniques. In future, this MLBC technique can be used with desired modification (viz. composition of processing fluid, speed and duration of cytospin etc.) to overcome the limitations of present study.

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