



Comparative study between Manual Liquid Based Cervical Cytology and Conventional Smear in Peri Menopausal and Post Menopausal Women

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Abstract

Objective: Aim of this study is to compare the conventional Pap smears with manual liquid based cervical cytology in peri menopausal and post menopausal women.

Method: A prospective study of 70 women was carried out in SGT University Hospital, Gurugram over the period of 1 year. Patients were randomly selected for pap smears and data collected was analysed.

Result: Analysis included patients aged more than 40 years. 70 cases were satisfactory for evaluation using LBC and 65 cases on conventional smear. Cytological abnormality was found in 29 cases on LBC and in 21 cases on conventional smear. Out of 70 cases 10 and 5 cases were diagnosed as ASCUS, 5 and 3 as HSIL, 12 and 8 as SCC on LBC and conventional smear respectively.

Conclusion: In conclusion, despite the smaller sample size MLBC method was found to be superior to the conventional smear.

Keywords: Conventional smear, Liquid based cytology, Bethesda.

Introduction

Cervical carcinoma is the second most common malignancy in women worldwide and the commonest malignancy in women in India. It accounts for 80% of deaths in developing countries like India.^{[1],[2]} Approximately 1,32,000 new cases are diagnosed and 74,000 deaths occur annually in India due to cervical cancer.^[3]

Screening programs using conventional Pap smear (CPS) have successfully reduced cervical cancer, but newer tests like liquid-based cytology (LBC- introduced in mid 1990s) have higher sensitivity than CPS^[4]

Peri and post menopausal age group poses a special challenge as far as screening by conventional pap smear is concerned. In post menopausal women there is tissue atrophy due to non estrogenic state following reduced ovarian function. This leads to decrease in the number of cells and thus increase in reporting of inadequate smears. Cervical discharge decreases in amount during menopause and may disappear completely after menopause. Thus it may be difficult to obtain cervical smears in post menopausal age group.^[5]

Also cytological pattern of the advanced atrophic menopause are influenced by dryness of the

genital tract and scarcity of recoverable cellular material, resulting in preparations that contain relatively few cells. The dominant squamous cells are of parabasal type. This may result in increased reporting of low grade squamous intraepithelial lesion (LSIL) or atypical squamous cells of undetermined significance (ASCUS) in smears, resulting in an increase in the false positive report. The mortality rate of cervical cancer can be significantly reduced if a woman is screened once when she is between the ages of 40-45 years.^[6]

In the last 15 years, new cytological techniques have been developed to improve the sensitivity of Pap smear.^[7] LBC leads to significantly fewer unsatisfactory smears (with associated financial costs and anxiety for women). In this the cells are spread out in a single layer which improves the rate of detection of the precursor lesions. It increases the detection of neoplastic lesions and reduces the over-diagnosis of benign disease processes.^{[7],[8]} Other advantages include provision of cells for detection of Human Papilloma Virus (HPV) DNA and other ancillary techniques like immunocytochemistry which can be performed on the residual sample.^{[2],[7]}

The present study aims at preparing cervical cytology smears in perimenopausal and post menopausal females using the manual method of liquid-based cervical cytology and to compare the results with that of conventional Pap smears according to Bethesda system of classification 2014^[9].

Materials and Methods

A prospective study of 100 cervical smears was carried out in the Department of Pathology, SGT University Hospital, Gurugram by conventional Pap smear and liquid-based cytology over the period of 1 year.

Study included cervical cytology samples from all women ≥ 40 years attending the OBG Out Patient Department with presenting complaints of white discharge per vagina, post-coital bleeding and for routine cervical cancer screening. Non co-

operative patients/ patients who do not gave consent, pregnant women, patients with massive bleeding per vagina were excluded from the study. Pap smears were taken from the cervix with endocervical cytobrush for both CS and MLBC. Smears were collected, processed and prepared as follows:

Patient was asked to lie down and her feet placed in stirrups to hold the feet in place during the examination. Speculum was inserted into the patient's vagina using an endocervical brush, sample was taken from the cervix by gently rotating the brush at 360 degrees. Sample collected was used to make thin smears on grease free glass slides. The remaining material from the brush was rinsed into a bottle containing liquid fixative for Manual Liquid Based Cytology. The sample was mixed, and then centrifuged at 2000 revolutions per minute for 5 minutes. At the end of the centrifugation process, the supernatant was decanted and a drop of the suspension was used to make a thin film on a grease free glass slide. Smears were then fixed in 95% ethanol. Smears were allowed to fix for 30 minutes and stained with the rapid pap staining technique.

Statistical Analysis

Statistical analysis was performed by the SPSS program for Windows. Continuous variables are presented as mean \pm SD, and categorical variables are presented as absolute numbers and percentage. Categorical variables were analyzed using the z test for proportion and chi square test. For all statistical tests, p value less than 0.05 was taken to indicate a significant difference.

Observations and Results

Table 1 Parity Wise Distribution of Cases

| PARITY | NO. OF CASES | CONVENTIONAL SMEAR | | LBC | |
|--------|--------------|--------------------|------------------|------------------|------------------|
| | | NORMAL | ABNORMAL | NORMAL | ABNORMAL |
| | | NO. OF CASES (%) | NO. OF CASES (%) | NO. OF CASES (%) | NO. OF CASES (%) |
| 1 | 3 | 3 (4.2%) | 0 | 3 (4.2%) | 0 |
| 2-4 | 63 | 45 (64.2%) | 18 (25.7%) | 38 (54.2%) | 25 (35.7%) |
| >5 | 4 | 1 (1.4%) | 3 (4.2%) | 0 | 4 (5.7%) |

Present study included 70 cases with age ranging from 40 to 65 years. It showed that no women was nulliparous and those having one child i.e 3 cases (4.2%) were found to be normal on both

conventional pap smear and LBC and all females (25.7% on CS and 35.7% on LBC) with abnormal pap smear were multipara.

Table 2 Chief Complaints Distribution of Cases

| CHIEF COMPLAINTS | NO. OF CASES (n=70) | % | NORMAL | ABNORMAL |
|-------------------|---------------------|------|--------|----------|
| White d/s pv | 30 | 42.8 | 30 | 0 |
| Blood stained d/s | 9 | 12.8 | 0 | 9 |
| PM bleeding | 21 | 30 | 2 | 19 |
| Greenish d/s | 3 | 4.2 | 3 | 0 |
| DUB | 2 | 2.8 | 1 | 1 |
| Curdy white d/s | 5 | 7.1 | 5 | 0 |

Chief complaints distribution showed maximum number of patients presented with white discharge per vaginum i.e 30 (42.8%), all of which were normal (i.e no epithelial abnormalities were

found); 9 cases (12.8%) presented with blood stained discharge and all were found to have abnormal pap smear.

Table 3 Adequacy Comparison of Conventional Smear and LBC

| ADEQUACY | CONVENTIONAL SMEAR (n=70) | LBC (n=70) | P-VALUE | RESULT |
|------------|---------------------------|------------|---------|-------------|
| Adequate | 65 (92.8%) | 70 (100%) | 0.01278 | Significant |
| Inadequate | 5 (7.1%) | 0 | 0.01278 | Significant |

Out of 70 cases 92.8% cases were adequate for evaluation and 100% cases were adequate on LBC. The difference regarding adequacy between

conventional smear and LBC was significant. (p value= 0.01278).

Table 4 Distribution of Type of Lesions on Conventional Smear and LBC

| LESIONS | CONVENTIONAL SMEAR (n=70) | | LBC (n = 70) | | P-VALUE | |
|-------------------------------|---------------------------|------|--------------|------|---------|-----------------|
| | NO. OF CASES | % | NO. OF CASES | % | | |
| NILM | 44 | 57.1 | 41 | 58.5 | 0.87288 | Not significant |
| Epithelial cell abnormality | 21 | 30 | 29 | 41.1 | 0.25014 | Not significant |
| Unsatisfactory for evaluation | 5 | 7.1 | 0 | 0 | 0.01278 | Significant |
| TOTAL | 70 | | 70 | | | |

The difference between NILM category and epithelial cell abnormality on conventional smear and LBC was not significant (p value= 0.87288 for NILM and 0.25014 for epithelial cell

abnormality). But significant difference was found between conventional smear and LBC in respect to unsatisfactory smear (p value =0.01254).

Table 5 Diagnosis Comparison

| DIAGNOSIS | CONVENTIONAL SMEAR | | LBC | | p - value | |
|--------------------------|-----------------------|------|-----------------------|------|-----------|-------------|
| | NO.OF CASES (n=70) | % | NO.OF CASES (n=70) | % | | |
| Inadequate Smear | 5 | 7.1 | - | - | 0.01278 | Significant |
| Inflammatory Smear– NILM | 19 | 27.1 | 13 | 20 | 0.26272 | NS |
| Atrophic Smear | 2 | 2.8 | 2 | 2.8 | 1.00000 | NS |
| Bacterial Vaginosis | 14 | 20 | 14 | 20 | 0.74896 | NS |
| Candidiasis | 5 | 7.1 | 6 | 8.5 | 0.75656 | NS |
| Trichomonal vaginitis | 4 | 5.7 | 6 | 8.5 | 0.29834 | NS |
| ASCUS | 5 | 7.1 | 10 | 14.2 | 0.15560 | NS |
| LSIL or LSIL Susupicious | 3 | 4.2 | 4 | 5.7 | 0.09692 | NS |
| HSIL or HSIL Suspicious | 5 | 7.1 | 3 | 4.2 | 1.00000 | NS |
| SCC | 8 | 11.4 | 12 | 17.1 | 0.47152 | NS |
| | | | | | 0.47152 | |

Although more cases of infectious agents like trichomonas, ASCUS and SCC were diagnosed on

LBC than on CS but the difference between the two methods was not significant.

Table 6 Diagnosis Comparison According to Peri and Post Menopausal Status

| LESIONS | CONVENTIONAL SMEAR | | LBC | |
|--------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| | PERI MENOPAUSAL CASES (n=33) | POST MENOPAUSAL CASES (n=37) | PERI MENOPAUSAL CASES (n=33) | POST MENOPAUSAL CASES (n=37) |
| Inadequate Smear | 2 | 3 | 0 | 0 |
| Inflammatory Smear– NILM | 12 | 7 | 9 | 4 |
| Atrophic Smear | 0 | 2 | 0 | 2 |
| Bacterial Vaginosis | 8 | 6 | 8 | 6 |
| Candidiasis | 3 | 2 | 4 | 2 |
| Trichomonal vaginitis | 2 | 2 | 4 | 2 |
| ASCUS | 2 | 3 | 2 | 8 |
| LSIL | 1 | 2 | 1 | 3 |
| HSIL | 3 | 2 | 1 | 2 |
| SCC | 0 | 8 | 2 | 10 |

On CS 2 cases (6%) were unsatisfactory in perimenopausal age group because of obscuring blood, inflammation and 3 cases (8.1%) were unsatisfactory due to postmenopausal changes.

More number of epithelial cell abnormalities were detected in postmenopausal females in comparison to perimenopausal age group.

Microphotographs

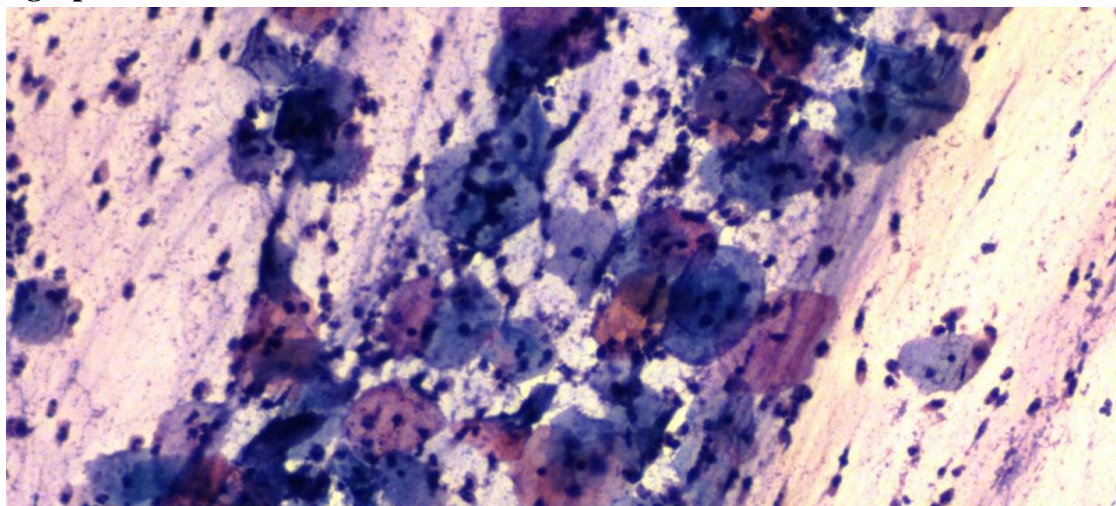


Figure 1 - Conventional pap smear. (Pap stain, 100X)

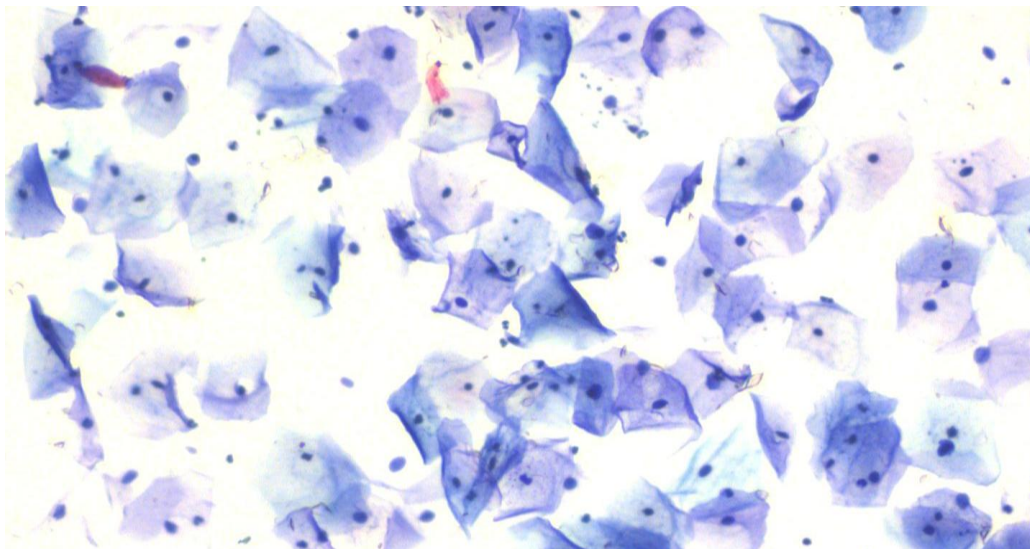


Figure 2 Liquid based cervical cytology. (Pap stain, 100X)

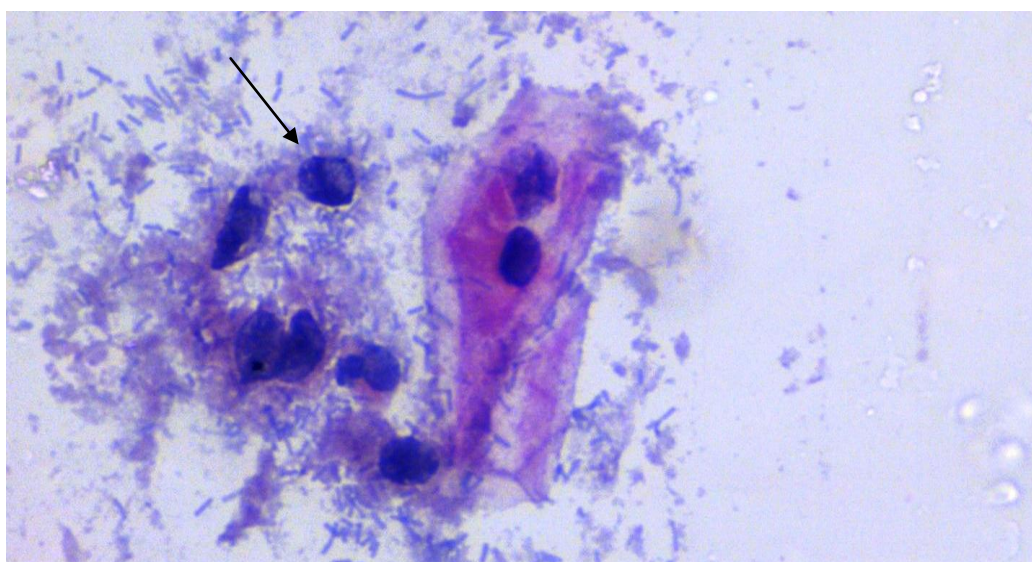


Figure 3 Trichomonal vaginitis (CS), pear shaped organism with eosinophilic cytoplasm. (Pap stain, 400X)

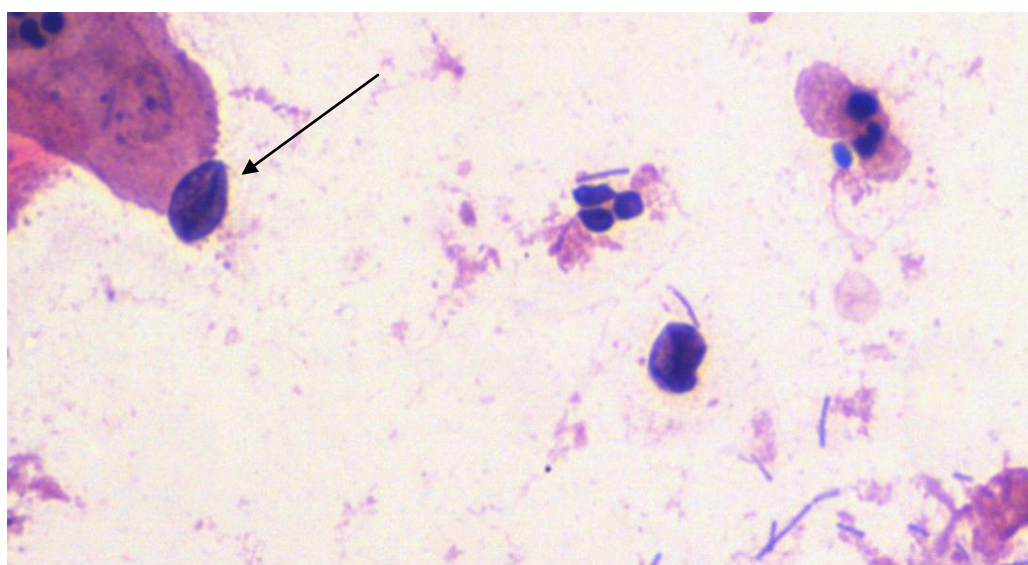


Figure 4 Trichomonal vaginitis (LBC), pear shaped organism with eosinophilic cytoplasm. (Pap stain, 100X)

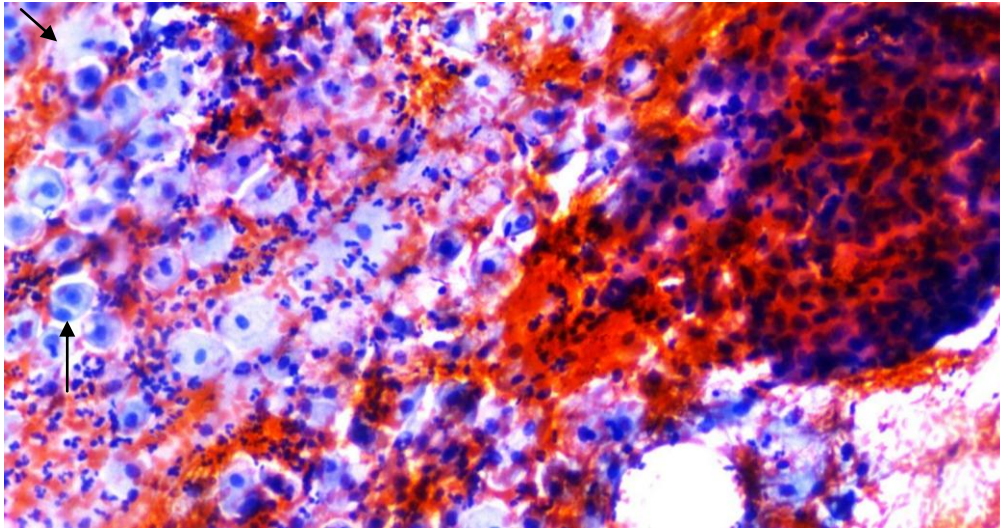


Figure 5 Atypical squamous cells of undetermined significance (ASCUS) (CS) shows atypical cells with binucleation and perinuclear halo in few. (Pap stain, 100X)

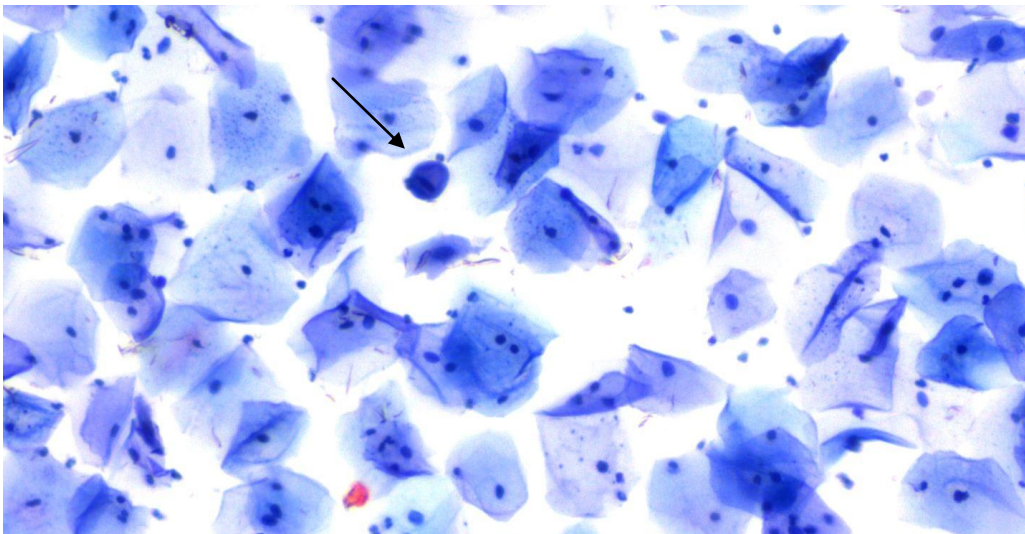


Figure 6 Atypical squamous cells of undetermined significance (ASCUS) (LBC) shows atypical cells with deranged N:C ratio. (Pap stain, 100X)

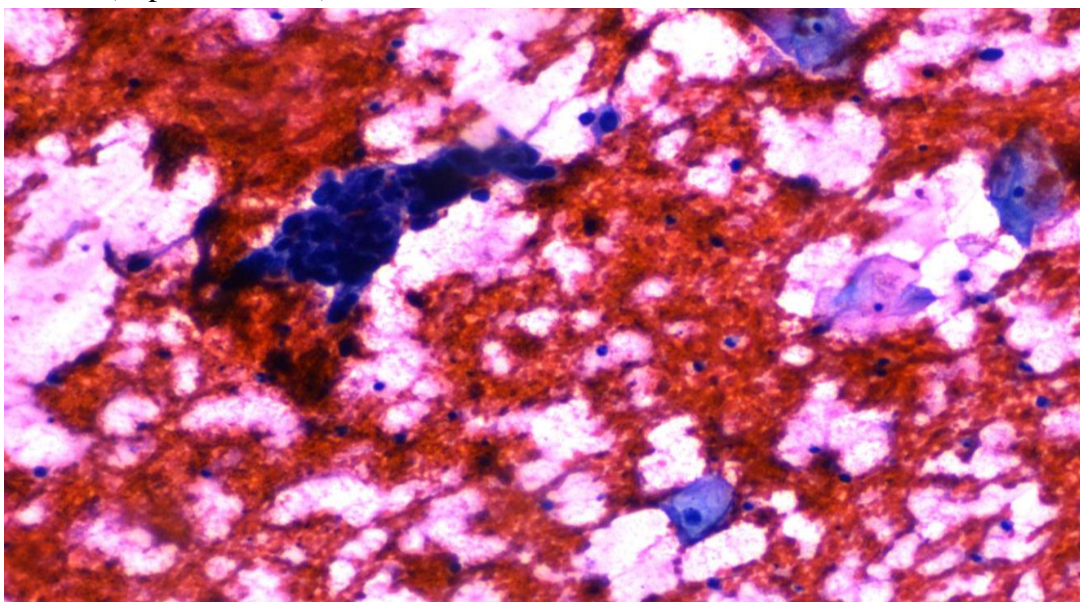


Figure 7 Squamous cell carcinoma (SCC) (CS) showing cluster of malignant cells with tumour diathesis and blood in the background. (Pap stain, 100X)

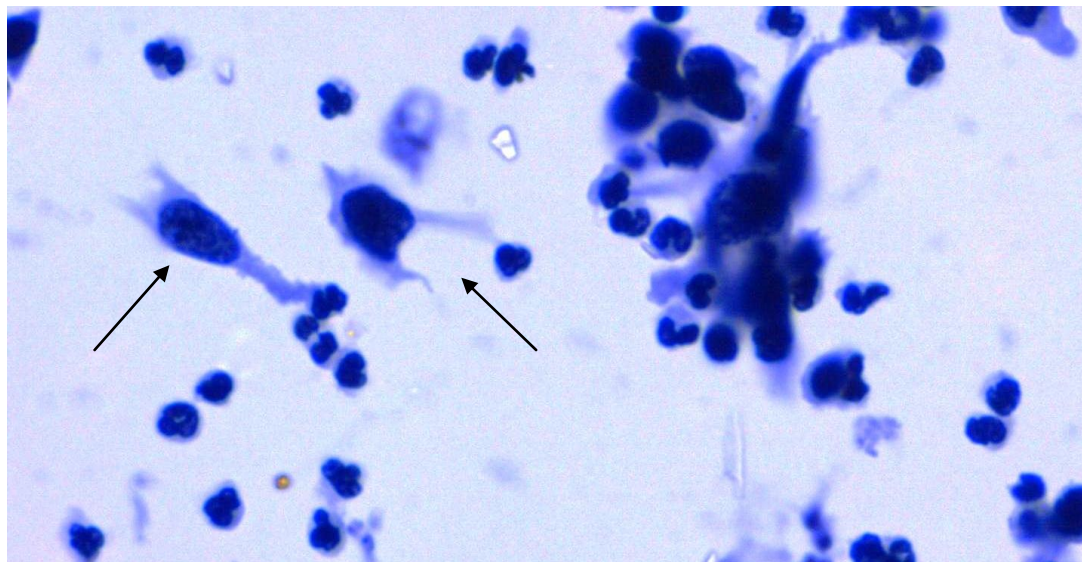


Figure 8 Squamous cell carcinoma (SCC) (LBC) showing malignant cells with marked pleomorphism, high N:C ratio, irregular nuclear contours, dense cytoplasm and prominent nucleoli with few tadpole cells. (Pap stain, 400X)

Discussion

The Papanicolaou 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. Liquid based cytology has been developed to address the sampling problems of conventional Pap smear.^[10] in which cellular structure is better preserved because the cells are immediately fixed. The process prevents drying artefacts and removes most contaminating mucus, red blood cells, bacteria and yeast.^[3]

In present study majority of the cases with abnormal cytology had multiparity, 29 and 21 abnormal cytology seen in LBC and conventional pap smear respectively; a finding concordant to the studies of Shankarnarayana et al^[11] and Parker et al^[12] who found that grand multiparity had a fourfold increased risk of developing cervical cancer.

The most common presenting complaint in present study was discharge per vaginum in 30 cases (42.8%). Kenneth and yao et al^[13] also had emphasized on the significance of vaginal discharge and its association with neoplastic changes in the cervix. All patients with post coital bleeding were found to have cytological

abnormality (24.2% on LBC and 18.5% on CS) and cervical carcinoma (17.1% on LBC and 11.4% on CS). These results are consistent with the known association of cervical neoplasia with post coital bleeding.^[14]

In this study, aim was to detect the best screening method for cervical cancer in peri and postmenopausal women who have challenges in the form of anatomical changes, less number of cells, and fewer secretions. It was observed that the percentage of satisfactory smears is higher with LBC (100 %) than Conventional smear (92.8 %) which was statistically significant. Also with increasing age, especially in post menopausal women, tissue atrophy occurs due to decline in estrogen levels leading to a poor yield of cells, ultimately increasing the unsatisfactory smears. In the present study it was observed that the increase in satisfactory smears and the decrease in unsatisfactory ones with the LBC method are likely due to the technique and the ease of the new cell collection device, the cytobrush. Drying artefacts were almost absent or minimal with liquid based cytology because of immersion of cells into the liquid fixative and specimen adequacy was greatly improved due to absence of limiting factors like blood, mucus and inflammatory cells. This was comparable to the

study done by Sherwani RK et al^[15] performed a similar study to evaluate liquid based cytology (Pap spin) and to compare the sensitivity of Pap spin with conventional Pap smear on 160 patients and concluded that 133 cases (83.1%) were satisfactory for evaluation on Pap spin and 51 cases (31.9%) on conventional Pap smear. Coste J et al^[16] also conducted a study which concluded that Conventional cervical smear tests were more often satisfactory (91% v 87%) which was opposite to the result of our study.

Microscopic details of infectious agents like trichomonas was enhanced on LBC which made it easy to detect (5.7% on CS and 8.5% on LBC) which was comparable to the study done by Chinaka CC et al^[10] and N Sherwani RK et al^[15] which also concluded that increase number of infectious agents were detected by LBC in comparison to CS. Although cases of bacterial vaginosis (14 cases on both CS and LBC) and candidiasis (5 cases on CS vs 6 on LBC) were almost similar in both LBC and CS.

The ASCUS is an intriguing, often confusing cytological entity in the management of cervical cancer. The false reporting of ASCUS is increased with the use of pap smear due to misinterpretation of inflammatory and reparative processes as ASCUS in smears. Postmenopausal changes result in an increase in reporting of ASCUS. Extensive inflammation, parabasal cells, organophilic cytoplasm, nuclear variations secondary to drying and degeneration associated with atrophic vaginitis may result in cellular changes that are falsely interpreted as ASCUS. This leads to overdiagnosis and overtreatment of many subjects. This study observed an increase in ASCUS cases when using LBC instead of CS (14.2% cases on LBC and 7.1% on CS). This is comparable with Cheung et al^[17] who detected an increase in the number of diagnoses by ThinPrep compared with CP (3.74% vs 3.19% for atypical squamous cells of undetermined significance (ASCUS). Also Maksem JA et al^[18] showed ASCUS were 3.3% in LBC vs 1.8% in CPS. Nonetheless, LSIL (4 cases each on LBC and 3

cases on CS) and HSIL (3 cases on LBC and 5 on CS) cases were found to be nearly equal between the two methods in the present study.

A limiting factor in this study is that it was not possible to assess the specificity and sensitivity of the techniques analyzed, because all patients were not evaluated by histopathology, which is used as the gold standard for diagnosis. However, Bolick et al^[19] reported sensitivity and specificity of liquid based cytology as 95.2% and 58% respectively whereas on conventional Pap smear, the same was 85% and 36% respectively. Beerman et al^[20] reported sensitivity and specificity of LBC as 96.2% and 98.2% respectively, whereas on conventional Pap smear it was 92.0% and 97.8% respectively.

The new cell collection devices for liquid-based cytology improved the amount of endocervical cells collected when compared with the classical spatula of the CS. Also, the liquid preservative vial in which the collected sample was stored and residual sample of LBC can be used for HPV DNA testing, for detection of Gonorrhoea and C.tracomatis. In this study it was observed that the liquid-based cytology tool may reduce obscuring inflammation, obscuring blood, and cytolysis resulting in more satisfactory slides. This is a potential advantage of the manual liquid-based technology over CS. Also the cells in MLBC are spread in a monolayer which allows better visualization when compared with the multilayer overlapped cells of CS.

MLBC has a very simple protocol and does not require any special imaging equipment thereby offering a simpler method with lower costs for cervical cancer screening. Other liquid based technologies, like Thin Prep TM and Surepath TM, require processor systems-automated equipment, plastic devices, filters and vacuums that are expensive and occupy valuable space in the laboratory. For this reason, these systems resulted in a high cost per slide.

Conclusion

In conclusion, despite the smaller sample size MLBC method was found to be superior to the conventional smear. Low estrogenic state poses a challenge for detection of cervical cancer by CS in postmenopausal females. LBC was found to be a better tool for cervical cancer screening. It has potential benefits like it offers a monolayer of cells generating enhanced visualization when compared with the multilayer overlapped cells of the CS, preservation of cells for ancillary techniques like HPV DNA testing. MLBC has a very simple protocol and does not require any special imaging equipment, thereby offering a simpler method with lower costs for cervical cancer screening. Hence in view of the advantages of MLBC, it would be worthwhile to further study this method as a cost effective alternative to the mechanized methods.

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