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Comparative Analysis of Conventional and Manual Liquid Based Cytology: A Study of 350 Cases

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Abstract: Pap smear is a screening procedure to detect precancerous lesions to prevent subsequent invasive cervical cancer. Manual Liquid Based Cytology (MLBC) has been developed as an alternative to Conventional Pap Smear (CPS) as it is said to increase the rate of detection of precancerous lesions as it reduces the screening time, the artefacts, giving a clean background on the smear. In this study, cervical screening was performed in all married women of reproductive age group who visited Department of Obstetrics and Gynaecology over a period of one year from February 2017 to January 2018. Out of which 350 cases were randomly selected for comparison between CPS and MLBC. Slides were examined for cytomorphological parameters. CPS showed higher percentage of inflammatory lesion 80% as compared to MLBC with 46.1%, Unsatisfactory smear in CPS was 10% and in MLBC was 49%, Normal smear was reported in 07% cases in CPS and 3.15% in MLBC. Most common cause of unsatisfactory smear in CPS is obscuration by blood and inflammation whereas that in MLBC was scant cellularity. 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, Unsatisfactory.

INTRODUCTION

Cervical cancer is one of the leading cause of mortality and morbidity among women worldwide and is the third most common cancer in women the world after first being Breast cancer and second colorectal cancer [1].

Since the introduction of cervical cancer screening studies in the western countries reported a reduction in both incidence & mortality of cervical cancer [2]. Several screening modalities are available for early detection of cervical cancer. In many developed countries, a decline in the incidence and mortality caused by cervical cancer has been observed in the past 30 years as a result of screening, early detection and treatment.

Cervical cancer screening has been performed for more than 50 years by the conventional scrape smears stained by papanicolaou (Pap) stain, which has substanstially reduced the incidence of cervical cancers, especially in developed countries [3]. In last 15 years, new cytological techniques have been developed to improve the sensitivity of Pap smear [4]. Liquid based cytology is the most accepted method, in which obscuring cells, mucus & blood are removed. It allows a better morphological assessment & improves the sensitivity of Pap smear. There is a lack of quality studies to compare the test performance of Manual Liquid Based Cytology (MLBC) and Conventional Pap Smear (CPS). Therefore, this study aims at comparing CPS and MLBC.

MATERIALS AND METHODS

The 350 cervico-vaginal smear samples of patients who attended the Department of Obstetrics and Gynaecology, over a period of 1year. The samples were taken as a part of routine hospital-based screening of patients for cervical lesion. 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, age less than 20 years, unmarried females and diagnosed cases of carcinoma cervix were excluded from the study.

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 Papanicoloau staining method.

OBSERVATION

350 random cases were selected for comparison by two method- conventional Pap smear and liquid based cytology (manual). Table-1 shows comparative analysis by techniques.

Out of total 350 smears, 282 (80%) smear were inflammatory by CPS and 163 (46.1%) smear were inflammatory by MLBC technique. 35 (11%) smear were Unsatisfactory by CPS whereas 169 (49%) smears were Unsatisfactory by MLBC technique.

Statistical analysis was performed using chi square test (x^2 test) where, $p<0.001$, i.e. highly significant correlation exists between two methods (Table-1).

Out of 282 inflammatory cases, 220 cases were found to be of nonspecific inflammatory pathology by CPS method while 62 cases were of specific inflammation (22%). However, by MLBC technique only 20 cases of specific inflammation were detected along with 143 cases of non-specific inflammation (Table-2). Statistical analysis was performed using chi square test (x^2 test) where, $p<0.001$, i.e. significant correlation exists between two methods (Table-2).

315 Of total 350 cases were satisfactory by CPS method but only 181(51%) cases were satisfactory by MLBC method while only 35(10%) cases were unsatisfactory by CPS and 169(48%) by MLBC technique (Table-3).

Table-4 shows cellular features are better in MLBC as compared to CPS. But cellular overlapping is present in MLBC technique which is less in CPS.

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 (Table-5).

Table-6 shows, the cellularity is adequate in most cases by CPS technique whereas MLBC showed less number of satisfactory smear while cellular distribution was better with MLBC technique.

Table-1: Distribution of Total Cases (N=350)

Smear	CPS	% Of Cases	MLBC	% of Cases
Inflammation	282	80	163	46.1
Normal	21	07	10	3.15
Unsatisfactory	35	10	169	49.00
Epithelial abnormality	12	03	8	1.75
Total	350	100	350	100

Table-2: Comparison of Specific & Nonspecific Inflammatory Cases by CPS (N=282) and MLBC Technique (N=163).

Techniques	Specific inflammatory cases	% of Cases	NonSpecific inflammatory cases	% of Cases	Total Cases of Inflammatory
CPS	62	22%	220	78%	282
MLBC	20	12%	143	88%	163

Table-3: Overall Comparison of Cps and Mlbc Technique (N=350)

Technique	Satisfactory cases	unsatisfactory cases
CPS	315(90%)	35(10%)
MLBC	181(51%)	169(49%)

Table-4: Comparison of Cellular Details in Conventional (CPS) and Manual Liquid Based Cytology (MLBC) Techniques

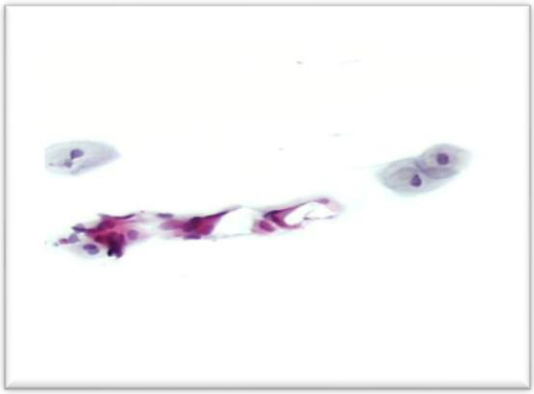
Cellular features	CPS	MLBC
Cellular overlap	Present(+)	Present(rare)
Sheets of cells	Larger	Smaller
Cell clusters	Frequently seen	Occasionally seen
Cellular elongation	Absent	Present
Nuclear detail	Good	Better
Membrane irregularity	Seen in more number of cases	Seen in less number of cases
Nucleoli	Preserved	Preserved
Cytoplasmic details	Good	Better

Table-5: Comparison of Background Details in Conventional (CPS) and Manual Liquid Based Cytology (MLBC) Techniques

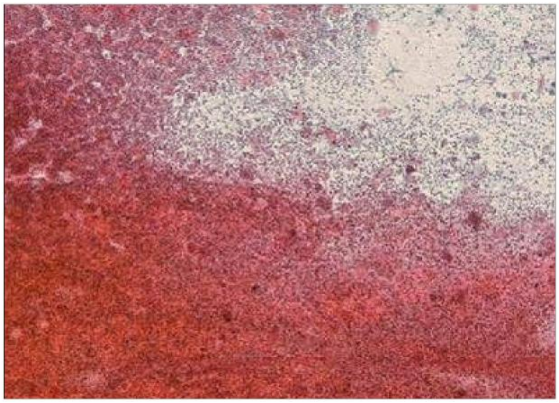
Background	CPS	MLBC
Clean	No	Yes
RBCs	Present	Absent
Neutrophils	Present	Absent
Necrosis	Diffuse	Clumped
Haemorrhage	Present	Present(rare)

Table-6: Comparison of Cytological Features in Conventional (CPS) and Manual Liquid Based Cytology (MLBC) Techniques

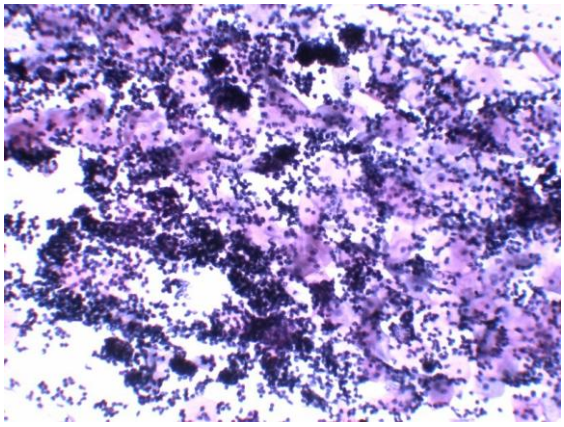
Cytological features	CPS	MLBC
Cellularity	Adequate	Inadequate
Cell preservation	Adequate	Adequate
Cell distribution	Uniform to uneven	Overlapping present
Cell size	Larger	Smaller
Architecture	Preserved	Preserved
Cytomorphology	Preserved	Preserved
Artifacts	Present	Present(rare)



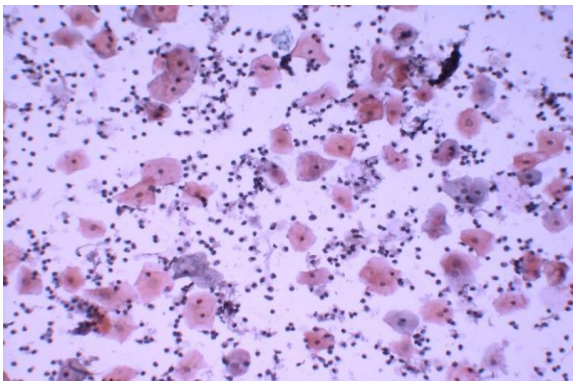
MLBC pap smear showing unsatisfactory smear due to scant squamous cell cellularity x200



4X view of conventional pap smear showing unsatisfactory smear due to obscuration by RBCs



10x view of Conventional Pap Smear showing obscuration by dense inflammatory infiltrate



10x view of LBC Pap Smear showing better cytomorphological detail in inflammatory smear

DISCUSSION

LBC is a technique which enables the cells to be suspended in a monolayer and thus improves the detection of precursor lesions by improving the specimen adequacy. It increases the histologically confirmed neoplastic and preneoplastic disease detection and thus improves the effectiveness of cervical cancer screening and also decreases the over diagnosis of the benign processes [5]. Controversy about its diagnostic accuracy prevails inspite of numerous studies and systematic reviews. MLBC is a cost effective method in low resource setting, which can be used as alternative method to much more expensive automated LBC [6].

Out of the 350 random samples were taken for comparison of two methods in which abnormal smear was detected in 12 cases (3%) by CPS and in 8 cases (1.75%) by MLBC. In contrast to present study, Sherwani *et al*., reported abnormality in 42 cases (26.2%) by Pap spin method, whereas conventional Pap smear detected abnormality in only 24 cases (15%) of total 160 cases [7]. Similar to our study in study by Garg *et al*., epithelial abnormality was reported in 2.89% cases by CPS and 1.75% by MLBC [8].

In the present study, only 21 cases of abnormal Pap smears were reported by CPS technique. Number of abnormal Pap smear got further reduced by MLBC (only 6 cases) and also density of atypical cell was

much less when compared to CPS. Similar findings were reported by Kawatkar *et al*., where 2 cases of HSIL were reported on CPS but were unsatisfactory by MLBC [9]. Various other studies also reported the same. In contrast Sherwani *et al*., [7] also reported that frequency of detection of abnormal Pap smear was higher with studies done by Hutchinson *et al*., [6] and Diaz-Rosario *et al*., [10] In the present study, reason for less number of cases showing epithelial abnormality could be because it was a split sample study, majority of cells were used in preparation of CPS slide.

Nandani *et al*., reported the number of unsatisfactory smears by CPS was 9 as compared to MLBC which had one unsatisfactory smear with scant squamous cellularity as observed by other studies In the present study, the number of unsatisfactory smears were more in MLBC as compared to CPS. Comparison of unsatisfactory smear rates have varied with some investigators reporting increased rates for liquid based methods reported by Maksem *et al*., [11] with some investigators reporting decreased as reported by Park *et a*l., [12] and some, no significant change.

Garg *et al*., reported 93% satisfactory smears by CPS but only 51.1% cases were satisfactory by MLBC. In 350 randomly selected cases, where both techniques (CPS and MLBC) were compared, number of satisfactory smears were much higher (315) by CPS as compared to MLBC (181 smear), which was in accordance with study by Monsonego *et al*., [13] In contrast Sherwani *et al.*, reported 133 cases (83.1%) smear satisfactory for evaluation on Pap spin and 51 cases (31.9%) on conventional Pap smear. Quite similarly, Weintraub and Morabia have reported an increased number of satisfactory cases (72.2% to 92%) on liquid based cytology than conventional smears. All drying artifact and cytolysis is 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. Kirschner *et a*l., [14] also reported the same.

As it is known, criteria for satisfactory smear are presence of transformation zone cells, absence of blood and mucus on smears. So, in the present study conventional smear were unsatisfactory only because of excess of blood and mucus while absence of endocervical cells and scant cellularity on MLBC (169 smears) was major cause of unsatisfactory smears. Similar findings were reported by other studies [11, 15, 16]. Reasons for unsatisfactory smears by MLBC technique in this study were:

- As it was split sample study and first portion of the sample was used to make conventional smear which have contained most of the endocervical components.
- Endocervical cells are more fragile than squamous epithelial cells, they might get disrupted during centrifugation of processing solution.
- Effect of preservative solution components on endocervical cells.

Nandani *et al*., reported clear background and well preserved nuclear details by MLBC. Similar was reported by other studies by Bollman *et al*., [17] and Bergeron *et al*., [16] In the present study, by MLBC technique, background was devoid of contaminating mucus, blood, excess of inflammatory cells, bacterias and also cytomorphological details were clear and well preserved.

Various parameters are also taken into consideration like cellularity, cleanliness of background, uniformity of distribution, artefacts, cellular overlapping, architectural and morphological changes and nuclear change. There are fewer morphological differences between LBC and conventional cytology than might be expected. There are several subtle general effects. The prompt fixation of LBC samples leads to good preservation and this is particularly seen in the clarity of presentation of chromatin. Nuclear membranes are also well visualized. In general, cells appear slightly smaller, due to rounding up effect of fixation in a liquid. The clarity of nuclear features is helpful in diagnosing dyskaryosis.

Cells showing low grade dyskaryosis, especially those with koilocytosis, are usually very easy to find and look identical to similar cells in CPS. High grade dyskaryosis (moderate to severe dyskaryosis) sometimes appear very similar to that seen in CPS. In LBC, severe dyskaryosis often presents as dispersed single cells. Hyperchromatic crowded cell groups are a problem in CPS and LBC cytology, though the reduced size of the groups and improved nuclear clarity does aid differentiation of benign and abnormal conditions. Severe dyskaryosis have dropped in frequency with LBC, since the specificity of this diagnosis for invasion has always been low. A diathesis can be recognized on LBC but has a different appearance to conventional smears, and nuclear features of poorly differentiated, non-keratinising tumors, are also well seen.

Dyskaryosis in LBC samples may be scanty, though usually it is not. The work of Mitchell & Medley defining the minimum number of abnormal cells on a conventional smear required for diagnosis has not yet been fully repeated for LBC.

CONCLUSION

Thus by undertaking the present study, it was concluded that frequency of inflammatory cervical lesion are more as compared to cases of epithelial abnormalities in Indian scenario. Comparative analysis of CPS and MLBC showed better results in cases of

CPS. However, in MLBC background was clean with better cytomorphological details but considering the satisfactory rate of smear, cellularity, cost effectiveness as well as simplicity of procedure it was concluded that CPS is better than MLBC in screening of cervical lesions. Split sample study could be partly responsible for low sensitivity of MLBC.

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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