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Original Research Article

Liquid Based Cytology Versus Conventional Cytology for Evaluation of Cervical Lesion - An Institutional Study

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Abstract:

Background: Cervical cancer is the second most common cancer in women following breast carcinoma. It is the cause of second most common cancer related mortality in women. As per Globocan 2020, 604100 new cases of cervical cancer were detected globally in 2020 and 341,831 deaths were attributed to the malignancy. In India, cervical cancer accounted for 9.4% of all cancers and 18.3% (123, 907) of new cases. The most commonly used and the most cost-effective screening test is cervical cytology and DNA testing for Human Papilloma virus in cervical cell samples. The clinical trials showed that these tests significantly decreased the morbidity and mortality in cervical cancer. Pap smear is the most cost-effective method for the prevention and detection of cervical cancer and has reduced the cervical cancer mortality by 70%. The imperfect sensitivity and variable smear quality of conventional Pap collection led to a search for a better alternative. In 1996, Liquid based cytology method was developed hoping to overcome the disadvantages of the previous method by improving sensitivity, sample adequacy and faster sample preparation. The present study was carried out to determine comparative effectiveness of conventional Pap smear and Liquid based cytology for evaluating cervical cytology over a period of two years from Nov 2020 – Oct 2022 in the Department of Pathology, M.K.C.G. Medical College and Hospital, Berhampur, Odisha.

Methods: The present study design is a prospective randomized evaluation of diagnostic tests. A total number of 220 subjects were studied over a period of 2 years (November 2020 to October 2022) at Department of Pathology, M.K.C.G. Medical College, Berhampur. Conventional Pap smears& Liquid based cytology were prepared from each sample collected (Split Sample Technique) and analyzed according to new BETHESDA System of reporting (2014).

Results: In the present study, comparison of morphological details and results of cervical cytology smears by both methods showed that, liquid-based cytology method provides less endocervical cells but higher clarity of background with reduced obscuring material (blood, mucus and inflammatory cells). The samples with liquid-based cytology were more representative, which allowed for better morphological evaluation. Also, more cases of epithelial lesions were detected by LBC and both sensitivity & specificity of epithelial abnormality detection was higher with LBC compared to conventional method.

Conclusion: Liquid based cytology requires more infrastructures and trained personnel, but is a better alternative to conventional smears for detection of premalignant and malignant conditions of cervix. Wherever economically feasible, LBC should be the first choice for cervical cytology collection.

Keywords: Liquid Based Cytology, Conventional cytology, Cervical Lesion.

Introduction

Almost 70% of the global burden of cervical cancer falls in lower socioeconomic groups and more than one fifth of all new cases are diagnosed in India. [1] Precancerous cervical lesions and cervical cancer are important public health problems especially in the developing countries. [2] The disproportionally high burden of cancer in developing countries is largely due to lack of cervical cancer screening programs. [3,4]

Frequently performed cytology screening programs have led to a decline in cervical cancer incidence and mortality in developed countries. In contrast, cervical cancer remains largely uncontrolled in high-risk developing countries because of ineffective or no screening program. [5] There are well structured tests to screen cervical cancer in the developed countries, but the healthcare infrastructure in the developing countries frequently does not support

cervical cancer screenithe developed screening programs at regular intervals, supported by a good vaccination program will save many lives. All of these issues have suggested the importance of cancer screening endeavors. [6,7]

The imperfect sensitivity and variable smear quality of conventional Pap collection led to a search for a better alternative. In 1996, Liquid based cytology method was developed hoping to overcome the disadvantages of the previous method by improving sensitivity, sample adequacy and faster sample preparation. [8,9] It was a new method of preparing cervical samples for cytological examination which involved making a suspension of cells from the collected sample and this is used to produce a thin layer of cells on a slide, unlike the conventional smear preparation. The new intervention could thus possibly form part of process of population screening to reduce the incidence of invasive cervical cancer. [10]

According to the literature, the LBC method decreased the rate of inadequate smears. [11,12] Though some researchers have reported no difference between the sensitivity of these two methods [13,14], others have supported LBC as having a higher sensitivity. [15,16]

Materials & Methods

The present study 'Liquid based cytology versus conventional cytology for evaluation of cervical Lesion' was carried out in 220 patients selected randomly from those patients with uterine cervical lesions attending the outpatient department of Dept of Obstetrics and Gynecology in M.K.C.G. Medical College & Hospital during a period of two years from Nov 2020 – Oct 2022.

Study Design: Prospective randomized evaluation of diagnostic tests.

Sample Size: 220 subjects.

Study Period: Two years (November 2020 – October 2022).

Study Setting: Department of Pathology, M.K.C.G. Medical College, Berhampur.

Ethical Approval: Taken from institutional ethical committee.

Inclusion Criteria: Women aged 20 to 70 years presenting with complaints of abnormal vaginal discharge, irregular periods, lower abdomen pain, post coital bleeding, or abnormal cervical findings on per speculum examination were studied randomly.

Exclusion Criteria: Women aged less than 20 years, pregnant women, women who underwent hysterectomy.

Methodology: After taking getting informed consent, a complete history was recorded and clinical examination was done, followed by collection of cytology specimen for both conventional Pap and Liquid Based Cytology.

Method of Collection of Sample: By putting the patient in dorsal position, per speculum examination was done, after visualisation of the cervix. A split-sample technique was used for collection of samples. Conventional Pap smear was collected by using a cyto brush and the cells were smeared on the glass slide. The glass slide was fixed immediately with 1:1 mixture of 95% ethyl alcohol and ether and then the slide was sent to the laboratory for processing, where the smear was stained with Papanicolaou stain and was reported.

For LBC same brush head was rinsed in preservative fluid, detached and suspended in preservative fluid after detachment. The liquid – prep collection (Celtra Zone) vial was capped, labelled (the patient's name and ID number on the vial, the patient information and medical history on the cytology requisition form) and sent to the laboratory for processing and analysis. In the lab the specimen was mixed well using a vortex for 5 to 20 seconds and the entire contents are poured into a clean 15 ml centrifuge tube and it was centrifuged for 10 minutes at 1500 RPM. After centrifugation supernatant fluid was discarded and to the residual pellet cellular base (this component is for encapsulation and adherence of processed cells on a clean glass slide) was added. The specimen was mixed using vortex and 50 micro litre pipetted onto a glass slide. The slide was allowed to dry, stained and was analyzed. (61)

Pap smear reporting was done according to the New Bethesda System 2014 for both.

In Liquid prep slide the cells are distributed over a small circular area which is 13 mm. In pap method, as the smear is manually prepared, the thickness of the smear varies. In LBC method, debris mucus, blood and cell overlap are largely eliminated. The number of leucocytes is low in LBC method. Hence the epithelial cells are easily visualised.

Results

Table 1: Comparison of satisfactory/ unsatisfactory rate between conventional pap smear and liquid based cytology

	Diagnostic Technique				P value
	Conventional Pap Smear		Liquid based Cytology		
	No.	%	No	%	
Satisfactory	170	77.28	210	95.46	0.02
Unsatisfactory	50	22.72	10	4.54	
Total	220	100	220	100	

In the present study, in LBC 92.73% cases showed satisfactory smears while in CPS 81.82% cases showed satisfactory results. Smear finding was compared between conventional pap smear and liquid based cytology using chi square test and significant difference was observed between conventional pap smear and liquid based cytology in the smear finding (p = 0.02).

Table 2: Comparison of cellularity between conventional pap smear and liquid based cytology

	Diagnostic Technique				P value
	Conventional Pap Smear		Liquid based Cytology		
	No.	%	No	%	
Cellularity	200	90.9	160	72.7	<0.0001
Scanty	20	9.1	60	27.3	
Total	220	100	220	100	

This table shows that good cellularity of smears was seen 72.8% cases of CPS and 36.3% cases of LBC. There was statistically significant difference of the smear cellularity between conventional pap smear and liquid based cytology (p<0.0001).

Table 3: Comparison of endocervical cells detection between conventional and liquid based cytology

Endocervical Cells	Diagnostic Technique				P value
	Conventional Pap Smear		Liquid based Cytology		
	No.	%	No	%	
Present	190	86.36	30	13.64	<0.001
Absent	30	13.64	190	86.36	
Total	220	100	220	100	

Comparison of endocervical cells detection in between conventional pap smear and liquid based cytology show, endocervical cells were present in 59.09% of cases in CPS while in LBC it was present in 40.91% cases. Comparing findings between conventional pap smear and liquid based cytology using chi square test, significant difference was observed in detection of endocervical cells (p < 0.001).

Table 4: Comparison of clarity of background between conventional pap smear and liquid based cytology

		Diagnostic Technique				P value
		Conventional Pap Smear		Liquid based Cytology		
		No.	%	No	%	
Inflammation	Present	180	81.81	40	8.19	<0.001
	Absent	40	18.19	180	81.81	
	Total	220	100	220	100	
Mucin	Present	160	72.72	60	27.28	<0.001
	Absent	60	27.27	160	72.72	
	Total	220	100	220	100	
Haemorrhage	Present	180	81.81	40	8.19	<0.001
	Absent	40	18.19	180	81.81	
	Total	220	100	220	100	

The above table shows that inflammatory cells (neutrophils) were present in 81.81% cases of CPS while in LBC it was 18.19%. Mucin was present in 72.72% cases of CPS which was found to be higher as compared to LBC (27.28%). Hemorrhage in CPS was found in 81.81% of cases while in LBC it was

18.19%. These finding suggest that in the present study clarity of background was found to be more for liquid-based cytology compared to conventional pap smear (Chi square Test, P<0.001).

Table 5: Diagnostic efficacy of CPS and LBC evaluation of cervical cytology

	LSIL		HSIL		SCC	
	CPS	LBC	CPS	LBC	CPS	LBC
Sensitivity %	72 .5	77.77	66.6	73.6	65.6	76.6
Specificity %	99.20	99.5	97.1	100	89.3	100
PPV	93.28	94.33	98 .2	100	92.2	100
NPV	99.59	90.92	94.93	94.66	33.33	44.44

PPV- Positive predictive value; NPV- Negative predictive value.

This table shows diagnostic efficacy of CPS and LBC for evaluation of cervical cytology. The sensitivity of CPS for detection of LSIL, HSIL & SCC were 72.5%, 66.6% & 65.6% respectively while that of LBC were 77.77%, 73.6% & 76.6% respectively. Specificity of LBC was 100% for all category of epithelial abnormalities except LSIL (99.5%), while in CPS it was 99.2% for LSIL, 97.1 % for HSIL & 89.3% for SCC.

PPV of CPS for LSIL lesions was 93.28%, 98.2% for HSIL & 92.2% for SCC, whereas PPV of LBC was 100% for all categories of epithelial abnormalities except for LSIL (94.33%). NPV of CPS for LSIL, HSIL & SCC were 99.59%, 94.93% & 33.33% respectively, while for LBC it was 90.92%, 94.66% & 44.44% respectively.

Table 6: Comparison of Sensitivity& Specificity of CPS & LBC methods (by taking LSIL as a cytology cutoff) between different authors

AUTHOR	YEAR	Sensitivity		Specificity	
		CPS	LBC	CPS	LBC
SontiSulochana et al	2014	72%	98%	88.8%	98.9%
Sueli Aparecida Batista et al	2015	74%	99.6%	87.7%	97.9%
Hawaldar Ranjana et al	2016	75%	99.4%	88.2%	97,4%
IknurAlkan Kusabbi et al	2017	77%	99.2%	88.6%	98.9%
Uma Singh et al	2018	81%	94%	95%	100%
Singh S et al	2019	80%	93.3%	92%	100%
Modi et al	2022	82%	95 .5%	94%	99.8%
Present Study	2022	72.5%	77.77%	94.93%	94.66%

In present study, both sensitivity and specificity of Liquid based cytology was higher than Conventional Pap smear. Similar observations were reported by other authors also. This indicates that, taking LSIL as a cut off, Liquid based cytology is comparatively better in detecting epithelial abnormalities than Conventional Pap smear.

Table 7: Comparison of Sensitivity& Specificity of CPS & LBC methods (by taking HSIL as a cytology cutoff) between different authors

Authors	Year	Sensitivity		Specificity	
		CPS	LBC	CPS	LBC
Shruti Singh et al	2019	42.8%	50%	100%	100%
Atif Hashmi et al	2020	41.6%	52%	100%	100%
A Premlata et al	2021	45.5%	53%	98.9%	99.9%
Modi et al	2022	47.5%	51%	98.7%	99.6%
Present Study	2022	65.6%	74.7%	93.8%	96.3%

In present study, sensitivity and specificity of Liquid based cytology was significantly higher than Conventional smear. Several authors found LBC to have more sensitivity, though many of them showed similar specificity.

Conclusion

Pap smear is one of the best available screening methods for early detection of cervical precancerous lesions. Liquid based cytology is an alternate technique for processing the cervical sample collected.

Most of the Western countries have switched over from Conventional Pap smear to Liquid based cytology even though the sensitivity and specificity are almost similar in various comparative studies. [13,14] The reason for this may be consistently reduced rate of unsatisfactory results on Liquid based cytology, more adequacy of smears, clarity of

background, improved sample processing and smaller area of screening. [11,12]

The present study was undertaken to study the difference between conventional and Liquid based cytology method in cervical pap samples and to assess diagnostic accuracy of Liquid based cytology in our setting.

In the present study, according to the Bethesda System, an adequate smear is defined as well defined/ well visualized squamous epithelial cells covering more than 10% of the slide surface. [17]

A smear that is satisfactory for evaluation is one in which squamous epithelial cells cover more than 10% of the slide surface and obscuring elements are less than 50-75%. A smear may be unsatisfactory for evaluation because of insufficient squamous cell component and when obscuring elements cover more than 75% epithelial cells. Different observations of our study show coherence & discoherence with previous studies. Some important studies & the number of cases in those studies are tabulated below.

In the present study, comparison of morphological details and results of cervical cytology smears by both methods showed that LBC method provides less endocervical cells but higher clarity of background with reduced obscuring material (blood, mucus and inflammatory cells). The samples with LBC were more representative, which allowed for better morphological evaluation. Also, more cases of epithelial lesions were detected by LBC and both sensitivity & specificity of epithelial abnormality detection was higher with LBC compared to conventional method.

Conventional cervical cytology (Pap smear) is a simple, cost-effective method of cervical cancer screening that has been in use for more than 50 years and is still a highly effective screening procedure. It is widely used because of easy method of preparation of slides and interpretation of results.

Liquid based cytology requires more infrastructures and trained personnel but is a better alternative to conventional smears for detection of premalignant and malignant conditions of cervix. Wherever economically feasible, LBC should be the first choice for cervical cytology collection.

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