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ORIGINAL RESEARCH

Conventional pap smear and liquid based cytology for cervical cancer screening: A Comparative Study

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Abstract

Aim: To compare conventional pap smear and Liquid based cytology for cervical cancer screening.

Material and Methods: The present prospective comparative study was conducted in the Department of Obstetrics and Gynaecology among women who are sexually active and reported to the OPD for gynecological consultation. Both conventional pap and liquid based cytology was performed on the same patient.

Results: Excellent correlation was found between LBC and Conventional pap smear w.r.t. inflammatory smear, atrophic smear, bacterial vaginosis, Atypical squamous/Glandular Cells, fungal infection (candidiasis), trichomonas vaginalis and SCC in this study.

Conclusion:Besides, the Liquid Based Cytology smears provided comparable cytomorphology with a clear and clean background. LBC can be a better alternative to conventional smear because of lower rate of unsatisfactory smears. Also, it has advantages of fewer less obscuring by blood, mucus material and inflammatory cells.

Keywords: Cervical cancer, Conventional Pap Smear, Liquid Based Cytology

Introduction:

Cervical cancer is a common cancer among women worldwide[1]. In developing countries it is the second most common cancer and third highest reason for death due to cancer after breast and lung cancer. Over 85 percent of new cases are diagnosed in resource-limited countries where it is the most common cancer in women after breast cancer[2]. Cervical cancer can have devastating effects with a veryhigh human, social, and economic cost, affecting womenin their prime. But this disease should not be a deathsentence, even in poor countries. Sexually transmitted human papillomavirus (HPV) infectionis the most important risk factor for cervical intraepithelialneoplasia (CIN) and invasive cervical cancer. Worldwide,the prevalence (%) of HPV 16 and/or HPV 18 amongwomen with normal cytology is 4.1%. HPV serotypes 16and 18 account for nearly 76.7% of cervical cancer in India.Globally, high-risk types HPV 16 and 18 contribute to morethan 70% of all cervical cancer cases[3].In India, cervical cancer is the second leading cause of new cancer cases and cancer-related deaths among gynaecological cancers in females, with an estimated 96,922 new cases and 60,078 deaths each years[3,4]. The available methods for screening are the Papanicolaou (pap) test (cytology) and Human Papilloma virus (HPV DNA) testing[5]. Screening can detect precursors and early-stage disease for both types of cervical cancer including squamous cell carcinoma and adenocarcinoma. Treatment for precursor lesions will prevent the development of invasive cervical cancer and thereby reduce cervical cancer related morbidity and mortality.In India, women do not have access to effective screening program and without major improvement in cytology services it will not be possible to screen even 25 percent of the population once in a lifetime in near future[6]. Cervical cytology was introduced by GeorgePapanicolaou into clinical practice in 1940[7]. In 1945, the Papanicolaou smear received the endorsement ofthe American cancer society as an effective methodfor the prevention of cervical cancer. Center of cytologyin Vancouver, British Columbia published data whichconfirmed that cytologic screening leads to a reductionin the rate of invasive cancer of the uterine cervix. Park et al[8] established that the sensitivity of theconventionalpap smears for the detection of cervical cancer precursors was less than 50%. Severallimitations of conventional smear were

identifiedincluding inadequate transfer of cells to slide[9], inhomogenous distribution of abnormal cells, presenceofobscuring blood, inflammation or thick areas of overlapping epithelial cells[10].Liquid based, thin layer technology was developed to address the limitation of Conventional pap smear. More than5,00,000 subjects have been studied with apreponderance of data indicating a significant benefitof liquid-based, thin layer technology in the detectionof cervical cancer precursor lesions and in theimprovement of specimen adequacy.Some advantages of LBC are the presence of100% of the collected sample a fixative liquid with thepossibility to perform cytochemical tests, molecularbiology test and new examinations, if required, using the samesample. Besides, there are less false-negative resultsand unsatisfactory smears. Also, cell preservation with asample of higher quality enables a better interpretation[11] and reduces the length of exams in 30%, therefore increasing the productivity of laboratories[12].In view of the emerging need of finding an effectivetool for detecting recurrence of cervical cancer, thisstudy was planned to assess the efficacy of LBC asa method for cytological follow up and detectionof cancer cervix andcompared with Conventional pap smear method to find thebestscreening method for detection of cancer in such patients.

Material and Methods: The present prospective comparative study was conducted in the Department of Obstetrics and Gynaecology among 200 women who are sexually active and reported to the OPD for gynecological consultation.

Inclusion Criteria:
1. Women in the age group of 21-65 years.
2. Patient willing and give consents for the procedure.

Exclusion Criteria:
1. Age < 21 and > 65 Years
2. Antenatal and Postnatal mothers (Puerperium Periods)
3. Previous screening test taken
4. Proven preinvasive lesions & on treatment
5. Menstruating women/Active vaginal Bleeding
6. Pervious surgery on cervix
7. Cases of HIV/STD
8. Utero cervical prolapsed

Study Methodology: A detailed history of the patient was taken and the patient was informed about the screening procedure. Informed consent was taken. Clinical data were collected from every woman,including age, menstrual status, any contraceptionmethod and any symptoms (discharge, vaginalbleeding, pelvic pain, and others). Both conventional pap and liquid based cytology was performed in the same patient.

Conventional pap smear (CPS): The smears were taken by a gynecologist in dorsal position under direct vision by usingCusco speculum and taken using withCytobrush from the Squamo-columnar junction. Cytobrushwasrotated against the ecto-cervix for a full rotation so as to include the transformationzone. Material from the Cytobrushwas spread onto a clean glass slide and fixed by bio spray (ethyl alcohol)for conventional method. They were then labeled and stained by the standardPapanicolaou method.

Liquid Based Cytology (LBC): The SurePath process begins withgynaecologist using a cervical brush (Cytobrush with detachable head) to collect agynecologic specimen. Rather than smearing cells collected by the sampling deviceson a glass slide, the heads of the sampling devices detach from the handle and areplaced into a vial of SurePath Preservative Fluid. The vial was capped, labeled, andsent with appropriate paperwork to the laboratory for processing. The heads of thesampling devices are never removed from the preservative vial containing the collected sample. In the laboratory, the preserved sample was mixed by vertexing and then transferredontoPrepStain Density Reagent. An enrichment step, consisting of centrifugalsedimentation through Density Reagent, partially removes non-diagnostic debris andexcess inflammatory cells from the sample. After centrifugation, the pelleted cells areresuspended, mixed and transferred to a PrepStain Settling Chamber.SurePath Preservative Fluid preserves cells for up to six months at refrigeratedtemperatures (2° C to 10° C) or up to 4 weeks at room temperature (15° to 30° C).Both the Conventional pap smear (CPS) and Liquid Based Cytology(LBC) smears were screened under low and high power. All the cases wereexamined for cytomorphological parameters. The details regarding cell

size,cytoplasmic and nuclear details were studied for making the diagnosis and were reported as per Bethesda System.

Bethesda System[13]: Cytologic reporting was done based on the NewBethesda System 2014 for both conventionalandliquid-based material.

a. Specimen type: whether conventional or liquid based.
b. Specimen adequacy: Conventional smear is considered adequate if there is at least 800–12000 well-preserved squamous cells.
c. LBC should have a cellularity of at least 5000 cells to beconsidered adequate.
d. Presence of transformation zone cells (endocervical cells or metaplastic squamous cells) is not considereda criterion for adequacy but should be reported ifpresent.
e. Presence of obscuring elements (blood, inflammatory cells, mucus, and others) that cover more than 75% ofthe conventional smear slide is an inadequacy criterion.
f. Cellularity assessment can be achieved by comparison with reference images or counting cells in a definednumber of fields at high power or low power.

Data was collected and subjected to statistical analysis using SPSS software.

Statistical analysis: Data so collected was tabulated in an excel sheet, under the guidance of statistician. The frequency and percentage of the measurements per group were used for statistical analysis (SPSS 22.00 for windows; SPSS inc, Chicago, USA). Reliability was assessed using Kappa Statistics. It is a statistical measure of inter-rater reliability for categorical variables. The inference is mentioned below:

values ≤ 0	no agreement
0.01-0.20	as none to slight,
0.21-0.40	as fair,
0.41- 0.60	as moderate,
0.61-0.80	as substantial
0.81-1.00	as almost perfect agreement.

Results: Out of 200patients, maximum patients (41%) were in 31–40-year age group followed by 21–30-year age group (26%) in the present study. Minimum subjects were from the age group of 61-65 years (7%) followed by 51-60 years. Majority of the subjects were from rural area (76%) in this study (table 1).

Table 1: Age distribution among the study subjects

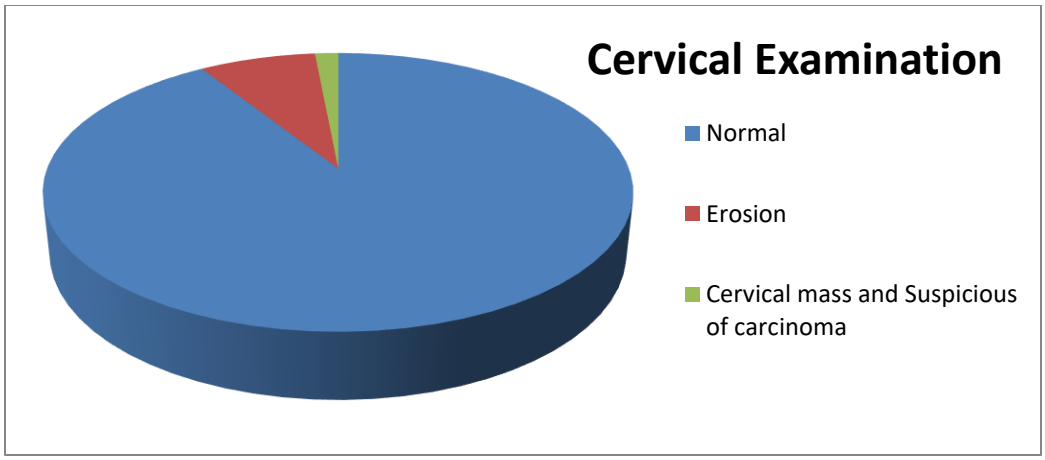
Age Group (in years)	N=200	%
21-30	52	26
31-40	82	41
41-50	36	18
51-60	16	8
61-65	14	7
Residential		
Rural	152	76
Urban	48	24

48% of the women came for routine health checkup. Most common clinical presentation among the study subjects was discharge per vaginum (16.5%) followed by menstrual irregularities (13.5%) and bleeding per vaginum (7.5%). Pain in lower abdomen and lower urinary tract symptoms was found in 6.5% and 5% of the women respectively (table 2).

Table 2: Clinical presentation among the study subjects

Parameters	N=200	%
Routine Health Check up	96	48
Menstrual irregularities	27	13.5
Dysfunction Bleeding per vaginum	15	7.5
Pain lower abdomen	13	6.5
Lower urinary tract symptoms	10	5
Dyspareunia	3	1.5
Discharge per vaginum	37	16.5
Mass protruding per vaginum	2	1
Infertility	2	1

Normal cervical examination was revealed in 91% of the women. Erosion and cervical mass with suspicious of carcinoma was reported in 7.5% and 1.5% of the women respectively (graph 1).



Graph 1: Cervical examination among the study subjects

LBC didn't reveal any unsatisfactory smear while PAP reported 14 unsatisfactory smears. Excellent correlation was found between LBC and Conventional pap smear w.r.t. inflammatory smear, atrophic smear, bacterial vaginosis, Atypical squamous/Glandular Cells, fungal infection (candidiasis), trichomonas vaginalis and SCC. Premalignant lesions (LSIL, HSIL) in the form of dyskaryotic changes were observed in pap smear i.e., LSIL 3%, HSIL 0.5% of the patients. 1 cases of frank malignancy were detected. The smear contained many malignant squamous cells with evidence of intracellular keratinization displaying high nuclear/cytoplasmic ratio, coarse chromatin with moderate amount of cytoplasm. Many fibre cells with hyperchromatic ovoid nuclei, which are diagnostic of invasive carcinoma were also observed (table 3).

Table3: Correlation between interpretation of conventional pap smear and LBC

Findings	Conventional pap smear	LBC	Kappa value
Unsatisfactory smear	14	0	0
NILM (Normal smear)	22	32	0.53
Inflammatory smear	136	130	0.81
Atrophic smear	8	11	0.79
LSIL	6	11	0.62
Bacterial Vaginosis	6	6	1
Atypical squamous/Glandular Cells	3	4	0.92
HSIL	1	2	0.50
Fungal infection-Candidiasis	2	2	1

Trichomonas Vaginalis	1	1	1
SCC	1	1	1

Name of author	LSIL	HSIL	SCC
Present Study: Conventional PAP	2.5%	0.5%	0.5%
LBC	4%	1%	0.5%
Bal M et al (2012)[17]	2.7%	0.7%	1.3%
Sachan P et al (2018)[18]	5.09%	0.48%	0.61%
Patel MM et al (2007)[19]	2.0%	0.1%	0.7%
Singh K et al (2015)[20]	0.8%	0.4	0.2%
Lakshmi P et al (2016)[21]	7.5%	6.0%	00
Mainali N et al (2018)[22]	3.75%	0.49%	0.2%
Sharma H et al (2021)[23]	1.3%	0.41%	0.2%

Discussion: The Conventional pap smear has been utilized for cervical cancerscreening for more than 50 years. Despite beingcredited with a 70% reduction in mortality for cervicalcancer, the false negative rate is still a cause forconcern. It is widely acknowledged that two third ofthe overall false negative rate can be attributed tosampling errors. Liquid based cytology has beendeveloped to address the sampling problems ofconventional pap smear[14].In the present study, 48% of the women came for routine checkup. Most common clinical presentation among the study subjects was discharge per vaginum (16.5%) followed by menstrual irregularities (13.5%) and bleeding per vaginum (7.5%). Pain in lower abdomen and lower urinary tract symptoms was found in 6.5% and 5% of the women respectively.Sherwani RK et al[14] in their study similarly reported that the most common presenting complaint in their study was discharge per vaginum (42.5%). Kenneth and Yao[15] have emphasized the significance of vaginaldischarge and its association with neoplastic changesin the cervix.Another study done by C Joshi[16],white discharge was found in 119 (59.5%) out of 200 women followed by complains of post coital bleeding (15.5%), irregular menstrual cycles (10.5%) and intermenstrual bleeding (10.5%).Our result is in approximate accordance with above mentioned studies. Conventional pap smear revealed unsatisfactory, NILM (Normal smear) and inflammatory smear among 7%, 11% and 68% of the subjects respectively. Atrophic smear, LSIL, bacterial vaginosis, atypical glandular cells, HSIL, fungal infection (candidiasis), trichomonas vaginalis and SCC was reported among 4%, 3%, 3%, 1.5%, 0.5%, 1%, 0.5% and 0.5% of the women respectively.LBC showed unsatisfactory, normal and inflammatory smear among 0%, 16% and 65% of the subjects respectively. Atrophic smear, LSIL, bacterial vaginosis, Atypical squamous/Glandular Cells, HSIL, fungal infection (candidiasis), trichomonas vaginalis and SCC was reported among 5.5%, 5.5%, 3%, 2%, 1%, 1%, 0.5% and 0.5% of the women respectively.

Our results are in accordance with above mentioned studies.
Results from other studies done bySaha D et al[24] (1.74%) and by Rawat et al[25] (1.3%)documents lower proportions.Variation in cytological abnormality observed among Indian studies might bedue to cultural differences, age of the individuals, incidenceof related infections, awareness about screening, and thepresence or absence of cervical screening programs indifferent parts of the country.LBC didn't reveal any unsatisfactory smear while PAP reported 14 unsatisfactory smears. Excellent correlation was found between LBC and Conventional pap smearw.r.t. inflammatory smear, atrophic smear, bacterial vaginosis, Atypical squamous/Glandular Cells, fungal infection (candidiasis), trichomonas vaginalis and SCC in this study. Sherwani RK et al[14] in their study similarly showed that satisfactory smears on Pap spin were83.1% as compared to 31.9% on conventional pap smears. Quite similarly Weintraub and Morabia[26] have reported an increased number of satisfactory cases(72.2% to 92%) on liquid-based cytology than conventional smears. All drying artefact and cytolysis are almost absent or minimal with liquid-basedcytologybecause of immersion of cells into the liquid fixativeand specimen adequacy was greatly improved due toabsence of limiting factors like blood, mucus andinflammatory cells. Only conventional smears wereunsatisfactory due to thick smear, which was not aproblem with liquid-based cytology due to evendistribution of cells.A study conducted in Japan examined the advantages of LBC over conventional pap smear in mass screeningfor cervical cancer. They reported that the detection rate of ASC-US and more severe lesions using LBC was1.44%, whereas it was 1.13% using conventional pap smear, hence a 1.3-fold higher detection rate when using LBC[27]. Our results showed comparable findings. Another study involving 310 cases comparedconventional pap smear with LBC and reported a significant difference in the inadequacy rate between thetwo methods. Unsatisfactory smears were noted to be 7.1% in the conventional pap smear compared to 1.6%in cases by LBC.

They did not find any significant difference in the disease detection rate[28]. Conversely, wefound a significant difference in the disease detection rate between conventional pap smear and LBC.Similarly, a prospective study including 312 cases of abnormal cervical cytology concluded that althoughLBC had a higher sensitivity of detecting epithelial lesions, overall there was no significant difference in theperformance of LBC compared with conventional pap smear[29].

Limitations: There were a few limitations of our study. First, the diagnostic accuracy of LBC was notevaluated in ourstudy as biopsy results were not available to calculate the sensitivity and specificity of LBC with respect tohistology. Second, hrHPV testing was not performed to determine the prevalence of hrHPV in patients withsquamous and glandular lesions on LBC. Therefore, we recommend large-scale prospective studies todetermine the diagnostic accuracy of LBC along with the prevalence of hrHPV in patients with glandular andsquamous lesions on LBC to have a better insight into the utility of LBC for cervical cancer screening in ourpopulation.

Conclusion: LBC smears were especially useful in hemorrhagic aswell as markedly inflammatory cases where the red blood cells and the inflammatorycells masked the morphology of the underlying epithelial cells in Conventional pap smears. Besides, the Liquid Based Cytology smears provided comparable cytomorphology witha clear and clean background.Other advantages of LBC method include possibility of multiple slides andancillary tests such as HPV DNA testing. There is a wealth of published literature showing increased sensitivity of LBC without loss of specificity. LBC reduces therate of unsatisfactory smears and the cells are better preserved and not obscured byblood, mucus, or inflammatory cells.LBC can be a better alternative to conventional smearbecause of lower rate of unsatisfactory smears.Also, it has advantages of fewer less obscuring by blood, mucusmaterial and inflammatory cells.Liquid based cytology is strongly advocated in thebest interest of public health, by improving the qualityof the sample and reducing the likelihood of falsenegative cytology results. Thus, it will significantlyimprove early detection and treatment of cervicallesions.

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