

Efficacy of Liquid-based Cytology vs Conventional PAP Smear in a Tertiary Care Hospital in Northeast Bihar: A Retrospective Study

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ABSTRACT

Background: This study was designed to compare the efficacy of two important screening modalities liquid-based cytology and conventional PAP smear. Both are cytological screening methods for cervical cancer.

Materials and methods: A prospective split-sample cervical cytological study was performed in 220 women attending Gynaecology Out patient Department at Mata Gujri Memorial Medical College, MG University, Kishanganj, Bihar over a period of 2 years (September 2021–September 2023).

Results: In a study of 220 conventional smears, 5.45% were unsatisfactory, while the majority (80%) were inflammatory. Among the 220 liquid-based cytology (LBC) smears, there were no unsatisfactory samples, and inflammatory smears were slightly less prevalent (78.18%). Dysplastic smears underwent human papillomavirus (HPV) testing, with 62.5% testing positive. Squamous intraepithelial lesions were more common in women over 40 and those with symptoms of vaginal discharge or an unhealthy cervix. Dysplastic lesions were more frequent in women who married early, had higher parity, or shorter intervals between deliveries. Neoplastic changes were associated with smoking and oral contraceptive pills (OCP) use. One human immunodeficiency virus (HIV) positive woman had carcinoma *in situ*.

Conclusion: The most common finding in cervical smear cytology was inflammation, followed by normal cytology. Liquid-based cytology showed a higher case pick-up rate (ASCUS, LSIL, CIS) and allowed for HPV detection, providing an advantage in screening. Most patients with neoplastic changes were HPV-positive. Overall, LBC was more sensitive and specific than conventional PAP smear (CPS), with increased detection rates of ASCUS, LSIL, and CIS due to obtaining more satisfactory smears and offering HPV detection.

Keywords: Cervical cancer, Conventional PAP smear, Liquid based cytology, Squamous intraepithelial lesions.

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INTRODUCTION

Commonly two types of carcinoma cervix are seen, the first and most common variety is epidermoid carcinoma. It arises from stratified squamous epithelium of the cervix and accounts for almost 80% of cancers in the cervix. The second variety endocervical carcinoma arises from the mucous membrane of the endocervical canal and accounts for 20% of all cervical cancer.

Histologically 95% of cervical cancers are squamous carcinoma and only 5% are adenocarcinoma. This is because the columnar epithelium of the endocervix often undergoes squamous metaplasia before undergoing malignant changes.

High-risk HPV infection is responsible for the majority of cancer. Human papillomavirus is now known to be the most common sexually transmitted infection at present. Among the high-risk HPV, genotype 16 is responsible for squamous cell carcinoma of the cervix, while HPV-18 is mostly responsible for cervical adenocarcinoma (Fig. 1).

Nearly 70% of cervical cancer patients present in India at the stages III and IV. Among 20% of women who develop cervical cancer die within the first year of diagnosis and the 5-year survival rate is 50% (Mittra et al.).¹

Before the actual development of the cancer cervix, there are changes in the region of the transformation zone, these changes can be picked up by cytology. These changes have been named cervical dysplasia, cervical intraepithelial neoplasia (CIN), and lately

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squamous intraepithelial lesions. Cervical dysplasia is a cytological term that was introduced by Water's and Regan to describe cells resembling cancer cells.²

The peak incidence of occurrence of dysplasia appears to be 10 years earlier than that of frank invasive cancer, this forms the basis of screening for cervical cancer.

Cervical cancer is the third largest cause of cancer mortality in India after cancers of the mouth, oropharynx, and esophagus (Fig. 2).



Fig. 1: Ayre's spatula and endocervical brush



Fig. 2: LBC vial and broom

This cancer affects poor women the most especially those living in rural areas, because they are less likely to get screened and treated. Risk factors for the development of cancer cervix include early age of marriage, early age of first pregnancy, multiparity, immunosuppression, dietary deficiency of vitamin A, increasing age OCP use, and smoking.

World Health Organization (WHO) recommends a triple intervention strategy (90-70-90)² in its venture toward the elimination of cervical cancer and to meet its target by 2030. The triple intervention strategy includes 90% of girls should be fully vaccinated with the HPV vaccine by 15 years of age, 70% of women should be screened using a high-performance test by the age of 35–45, and 90% of women identified with cervical disease should receive treatment.

There are various screening procedures for the CA cervix-cervicography, visual inspection (VILI, VIA), cervical cytology, HPV testing and colposcopy, digital cervicography, and smartoscopy (where smart phones are utilized to evaluate the cervix). Among these cervical cytology is the most widely used screening test. It includes both conventional Papanicolaou test (PAP) smear and liquid base cytology.

- Both LBC and CPS detect koilocytes, with LBC HPV detection is also possible.
- So, to meet needs of the hour my study is designed to observe the changes in cervical cytological smears of women taking into account various associated risk factors and to compare the efficacy of two important screening modalities conventional PAP smear and liquid based cytology.

Aims and Objective

- To compare the efficacy of conventional PAP smear and liquid-based cytology for cervical cancer screening.
- To look for the incidence of HPV infection among women with dysplastic changes of the cervix.

MATERIALS AND METHODS

This study was conducted in the Department of Obstetrics and Gynaecology at Mata Gujri Memorial Medical College, MG University, Kishanganj, Bihar with an objective to compare the efficacy of conventional PAP smear with liquid-based cytology for screening of pre-malignant and malignant lesions of the cervix.

The study population included 220 women visiting the Out patient Department of Obstetrics and Gynaecology.

Period of Study: September 2021–September 2023.

Inclusion Criteria

All sexually active women or women who were sexually active any time during their past life aged between 21 and 60 years were included in this study.

Exclusion Criteria

In clinical research and health screening protocols, certain categories of women are typically excluded to ensure the accuracy and safety of the study. These exclusions generally include women younger than 21 years or older than 60 years, as these age-groups may present unique physiological characteristics or risk factors that could skew study results. Women currently menstruating or experiencing any form of active vaginal bleeding are also excluded to avoid confounding factors that could affect diagnostic procedures, such as PAP smears. Additionally, women who have undergone hysterectomies or have been diagnosed or treated for carcinomas are excluded due to their altered reproductive anatomy or ongoing cancer treatment, which could impact study outcomes.

Further exclusions apply to women who have never been sexually active, as certain screening methods, like PAP smears, are less relevant to this population. Women with any observable frank growths in the vaginal area are excluded to prevent misinterpretation of results, as such growths can complicate the diagnostic process. Those who have been recently screened within the last 3 years with a negative PAP report is generally excluded to avoid redundancy and over-screening. Similarly, women who have undergone ablative treatment or local excision for CIN II or III within the last 3 months are excluded to allow sufficient time for tissue healing and to avoid confounding post-treatment changes with study findings. These exclusion criteria are essential to maintain the integrity of the research and ensure that the findings are applicable and safe for the target population.

(* Ablative treatment includes Cryosurgery, Electrocoagulation, Laser Ablation*)

(* Excisional procedure like LLETZ, LEEP, NETZ, Cone Biopsy*)

All samples were split and for PAP smear samples were sent to Pathology Department and for LBC samples were sent to JB Laboratory (private lab) in Kishanganj.

Verbal informed consent was obtained from all participants after detail explanation of the procedure.

General Instructions

Before obtaining an ideal PAP smear specimen, patients were instructed to avoid vaginal medication or douching for 48 hours before the procedure and sexual abstinence for a night before the appointment. Smears were never taken during menses and patients were called at later dates after their menses were over.

- The aim of taking a sample was explained to women and consent was taken.
- Patients' complaints if any were noted.
- Details history was taken under the following heading along with per speculum examinations:
 - Present age.
 - Age at marriage.
 - Parity.
 - Age at first childbirth.
 - Interval between deliveries.
 - H/O OCP use.
 - H/O of HIV or any STD.
 - H/O smoking or chewing tobacco.
 - C/O or H/O exclusive vaginal discharge.

PROCEDURE

Samples were always collected before the application of Lugol's iodine or acetic acid (if in any case, we wanted visual inspection or biopsy), or before doing bimanual examination.

{***Special circumstance:** PAP smear in postmenopausal women is inaccurate and often negative on account of indrawing of the squamocolumnar junction, dry vagina, and poor exfoliation of cells. This can be improved by administration of estrogen cream/oral estrogen daily for 7–10 days and then taking smears*}.

The patient was placed in a dorsal position, the labia were separated, and the Cusco's self-retaining speculum was gently inserted without the use of lubricant or jelly. The cervix was exposed and visualized for any gross pathological features under adequate light and findings were noted.

Samples were always collected in labelled vials (before taking sample vial was labelled with patient name, age and registration no.).

Liquid-based cytology broom was inserted such that the longer central bristles were in the endo cervical canal and then the broom was rotated 5 times in the same direction for 360°. First bristle was smeared over a glass slide for PAP smear which was then quickly cyto-fixed by spraying aerosol (95% ethyl alcohol) over it or by immersing the slide in a fixative for 30 minutes and sent to Pathology Department for staining with Papanicolaou stain and examination under microscope.

Later head of the broom was detached from the liquid-containing vial and sent for a cytological smear (vial contains buffered methanol solution).

For LBC, the vial was placed into the thin prep processor along with a disposable gynecological filter. The instrument homogenizes the sample by spinning the filter. Shear forces created during spinning break up blood, mucous, and debris keeping the true cell clusters intact.

Then the liquid preservative solution was filtered through a membrane filter with a pore size specially designed to trap epithelial cells while allowing contaminating red blood cells and inflammatory cells to pass through.

The epithelial cells collected on the membrane filter are then transferred on a glass slide in the circle of 20 mm diameter. After that, the slides are dried and stained by an automated stainer. This produces a relatively thin monolayer type of preparation.

Later remaining liquid can be used for HPV testing.

As HPV testing is expensive, we requested SR Laboratory to preserve the remaining liquid after preparation of cytological smear for all samples but to perform HPV testing only for those smears that showed either ASCUS, LSIL, HSIL, or CIS.

Bethesda System 2014 was used for reporting cervical cytology.

Reports were compared as per criteria mentioned earlier in detailed history taking and per speculum examination findings.

RESULTS

- Most of the participants were in the age-group of 31–40 years (120 women) 54.54%, women between the age-group of 41–50 years were 56 (25.45%), women between age 51 and 60 years were (11.82%) and women between 21 and 30 years were 18 in number (8.18%).
- Among 220 women, the majority 142 (64.54%) women were married at <20 years of age, while 78 women (35.46%) were married at ≥20 years of age.
- The majority of women (116/220), i.e., 53.70% had their first child before the age of 20 years.
- Out of 220 women, 60 women had a parity of ≤2, and 160 women had a parity of ≥3.
- Out of 220, 26 women (11.82%) were OCP users and 11 were smokers (5%).
- 142 women (64.54%) out of 220 women complained of excessive vaginal discharge.
- 137 women (62.27%) out of 220 women were found to have clinically unhealthy cervix on per speculum examination.
- Among them, some had both the above findings.
- Out of 220 conventional smears there were 12 (5.45%) unsatisfactory smear, 16 normal smears (7.27%), 176 (80%) inflammatory smears 6 (2.73%) atrophic smears, 2 ASCUS (0.91%) smears, 4 LSIL (1.82%) smears 2 HSIL (0.91%) smears and 2 CIS smears (0.91%).
- Among 220 LBC smear there were 0 unsatisfactory smears (0%), 18 normal smears (8.18%), 172 (78.18%) inflammatory smears, 6 (2.73%) atrophic smears, 10 ASCUS smears (4.54%), 8 LSIL smears (3.64%), 2 HSIL smears (0.91%) and 4 (1.82%) CIS smears.
- All 24 women with dysplastic smears underwent HPV testing by LBC method among them 15 (62.50%) were found to be infected with HPV.
- Squamous intraepithelial lesions were more common over 40 years of age.
- More number of abnormal smears were observed among patients with c/o vaginal discharge or unhealthy cervix (seen by P/S examination). More number of dysplastic lesions were

observed among people who were married early, and had higher parity or decreased interval between deliveries.

- Neoplastic changes were among smokers and OCP users.
- There was only one HIV positive woman out of 220 women and she was found to have carcinoma *in situ*.

DISCUSSION

The cytological diagnosis of cervical smear has become very important screening test for the detection of pre-invasive and invasive cervical epithelial abnormality. Screening of the female population for cervical neoplasia is a simple and reliable method that greatly reduces the mortality and morbidity associated with CA cervix if detected in its pre-invasive stage.

This study was planned to observe the changes in cervical cytology and compare the efficacy of conventional PAP smear and LBC.

Study group comprised 220 women who were observed for various characteristics like age, age at marriage, age at first childbirth, parity, interval b/w deliveries, H/O HIV, H/O use of OCP, and H/O smoking and then the association of this characteristic with respect to various types of lesions were observed.³

The study group comprised of women between age 21 and 60 years. majority of screened women were in the age-group 31–40 years (54.54%), this was followed by age-group 41–50 years (25.45%).

More abnormal smears were detected in higher age-groups (ASCUS–9.75%, LSIL–7.32%, HSIL–2.44%, CIS–2.44%) in comparison to the lower age-group (ASCUS–1.45%, LSIL–1.45%, HSIL–0%, CIS–1.45%). This difference is statistically significant with p -value <0.00001.

In a study by Thomison et al.⁴ 20% of cervical cancer were detected in the perimenopausal age-group which is concordant with my study.

Similarly in a study conducted by Ranabhat et al.,⁵ 80% of abnormal smears (epithelial lesions) was found in the age-group above 40 years.

In a study by Christine Bekos et al.⁶ it was found that HSIL and LSIL were more common in higher age-groups as younger patients had higher rates of regression, complete remission, and lower rates of progression.

Mishra et al.⁷ in 2009 found that 51.5% of squamous intraepithelial lesions and 75.3% of cancer cases were detected in women above 40 years, which is concordant with this study.

Similarly, in a study by Sherwani RK et al.,⁸ 48.1% of screened women were in the fourth decade of life, and cases of LSIL were found mostly in 4th decade of life.

My study showed 02 cases of CIS (1.45%) at the younger age-group (<40 years), a finding in contrast to studies by Parker et al.⁹ who reported carcinomatous cases beyond 70 years. My observations may be due to the common practice of early marriage in this part of the country and there is no practice of screening.

Mayavati Mhaske et al.¹⁰ in their study of risk factor association and cervical dysplasia showed the association between early age of marriage and CA cervix, which corresponds with the present study.

Another study by Sreejata Raychaudhuri and Sukanta Mandal¹¹ showed that early age of marriage indicated an early exposure to sexual activities, a leading risk factor for CA cervix which corresponds with the current study.

A study over time since first sexual intercourse and the risk of cervical cancer development by Martyn Plummer et al.¹² states that first cervical infection with HPV often occurs soon after first sexual

intercourse is a reasonable proxy for early age at first exposure to HPV and finally progress to CA cervix. So, this study also corresponds with that of ours.

In my study, most of the women (53.70%) had their first child before the age of 20 years. Among those who had their 1st child before 20 years of age, 6.30% had ASCUS, 5.17% had LSIL, 1.72% had HSIL, and 2.59% had CIS. While among those who had their first childbirth \geq 20 years of age 3% had ASCUS, 3% had LSIL, 0% had HSIL and 1% had CIS. The p -value is 0.02642 which is statistically significant, i.e., neoplastic changes were more common among those who had their first child before 20 years of age.

In our state where illiteracy is prevalent added upon by lack of awareness leads to an early age of marriage, followed by early consumption, and then to early age of first childbirth. this series of events leads to an increased risk of CA Cervix.

My study corresponds to a study by KS Louie et al.¹³ for the International Agency for Research on Cancer, which shows that the early age of first sexual intercourse and early pregnancy are a risk factors for CA Cervix in developing countries.

In my study, most of the women (73.72%) had parity \geq 3, and only 27.27% of women had parity \leq 2. This may be due to a lack of awareness of various contraceptive majors provided by the government and the benefits of small families.

Among those who had parity \geq 3, 3.75% had normal smears, 83.74% had inflammatory smears, 3.75% had ASCUS, 3.75% had LSIL, 1.25% had HSIL and 1.88% had CIS.

Above mentioned data shows that higher parity leads to a greater number of abnormal smears than lower parity which is concordant to the study of Shankarnarayana et al.¹⁴

A study by Mishra et al.⁷ showed that the frequency of both premalignant lesions and cancer is more with increasing parity, which was similar to my study.

This study is also concordant with that of Parkin DM et al.¹⁵ who concluded in their work that 07 or more full term pregnancies had a 4-fold increase in the risk of developing squamous cell carcinoma of the cervix.⁴

Limitations

- Loss of follow-up.
- LCB was not available in the hospital.
- Poor patients don't want to be tested in a private lab.

CONCLUSION

- A most common finding in cervical smear cytology was inflammation followed by normal cytology.
- Case picks rate (i.e., ASCUS, LSIL, CIS) was more with LBC.
- Through the LBC method HPV detection is possible, which adds an extra edge of screening.
- Maximum number of patients with neoplastic changes were HPV-positive.

So, it can be concluded that the LBC modality appears more sensitive and specific than CPS with an increase in detection rates of ASUS, LSIL, and CIS by virtue of getting more satisfactory smears and with the added advantage of HPV detection.

Ethical Approval

Approved by Ethical Communities.

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