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**Original Research Article**

# A prospective study of cervical lesions diagnosed by liquid based cytology in Western Rajasthan, India population

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## ABSTRACT

**Background:** Carcinoma cervix is the second most common malignancy of women in India after breast cancer. The present study was conducted to determine the spectrum of cervical lesions by liquid-based cytology in Western Rajasthan population.
**Methods:** It is a Prospective study on 1087 cervical samples carried over a period of 1 year. Cervical samples were taken and processed by SurePath™ LBC.
**Results:** Of total 1087 cases 959 were negative for intraepithelial lesion or malignancy (88.22%). 88 cases (8.09%) were reported as unsatisfactory. Among the non- neoplastic cases- bacterial vaginosis was reported in 209 cases (21.8%), *Candida* in 77 cases (8.02%), both *Candida* and bacterial vaginosis in 12 cases (1.25%), reactive cellular changes in 193 cases (20.12%), and *Trichomonas vaginalis* in 01 case. Among pre-malignant and malignant lesions, 40 cases (4.17%) the distribution was as follows-atypical squamous cells of undetermined significance 16(1.67%), atypical squamous cell-cannot rule out high grade 08 cases (0.83%), Low grade squamous intraepithelial lesion 04 cases (0.42%), high grade squamous intraepithelial lesion 07 cases (0.73%), Atypical glandular cell favoring neoplastic 01 case (0.15%), and squamous cell carcinoma 04 cases (0.42%). Histopathological co-relation of premalignant and malignant lesions was further studied.
**Conclusions:** Liquid based cytology is an effective screening and diagnostic procedure for cervical abnormalities. Among pre-malignant and malignant lesions, histo-pathological correlation increased with increased grade of severity of lesions. To the best of knowledge, this is the largest study of liquid based cytology in the Western Rajasthan.

**Keywords:** Cervical cancer, Liquid based cytology, Western Rajasthan

## INTRODUCTION

In India, cervical cancer is the second most common cancer in women aged 15-44 years after breast cancer, accounting for almost 14% of all female cancer cases. More than 80% of new cervical cancer cases occur in developing and underdeveloped countries.[1-3] India has the largest burden of cervical cancer patients in the world. Almost 70% of the global burden of cervical cancer falls in areas with lower levels of development, and more than one-fifth of all new cases are diagnosed in India.[3] In its advanced stages, it has devastating outcomes in terms of both prognosis and quality of life, with approximately 67,477 deaths (23.3% of all cancer-related deaths) each year in Indian women.[2,4]

Western Rajasthan, being a relatively underprivileged part of India, suffers from high rate of illiteracy, lack of mass screening programmes and public awareness, child marriages, poor medical infrastructure and different

cultural, climatic and geographical profile as compared to the rest of the country. Hence, it becomes increasingly important for implementation of regular cervical screening programmes. Liquid Based Cytology (LBC) is now the standard procedure for carrying out cervical smear examination and the Bethesda system of reporting for cervical cytology offers a standardized system for reporting of gynecological cytology. The present study was undertaken to study the distribution and spectrum of cervical lesions in Western Rajasthan population. To the best of our knowledge, the present study is the largest and first study of cervical liquid-based cytology in patients visiting a tertiary care hospital in Western Rajasthan.

The study was carried out with the primary objective of finding the overall prevalence and distribution of various cervical non-neoplastic and Intra-epithelial neoplastic lesions in liquid-based cytology in Western Rajasthan population.

Histopathological correlation of Intra-epithelial lesions was done, where-ever available.

## METHODS

The study was carried at a Tertiary care institute of Western Rajasthan (AIIMS, Jodhpur) over a period of 1 year (October 2016- September 2017). A total of 1,087 cases were included in the study. After approval from Institutional Ethics Committee, the study was conducted on the patients attending the indoor wards and OPD of the departments of Obstetrics and Gynecology. An informed consent was taken from every patient included in the study.

### *Inclusion criteria*

- Women between 20 and 75 years of age were included in the study. The patients presented with varied complaints of vaginal discharge, post-coital bleeding, menstrual irregularities, pruritus, infertility, dyspareunia and dysuria.

### *Exclusion criteria*

- Pregnant women
- Women with active vaginal bleeding
- Hysterectomized women, and women with frank growth.
- IUD users
- Women age >75 and <20 years

A detailed proforma including all basic information and details about complaints was filled out for each patient. At the beginning of the examination, a cytobrush was introduced into the external cervical os and scraped to collect cells from ectocervix and endocervix. The brush head was detached and suspended in LBC vial containing preservative fluid, which was transferred to the cytopathology laboratory. LBC (Sure Path) cervical samples were processed according to manufacturer instructions. One slide was prepared from each case which was stained with Papanicolaou stain. All the LBC smears were examined by light microscopy.

Slides were reported and assigned a category according to the Bethesda system 2014. Wherever available, the results of cervical Pap samples were correlated with follow-up cervical biopsies/resection specimens.

## RESULTS

### *Unsatisfactory smears*

Out of a total of 1087 smears, 999 smears (91.9%) were satisfactory for evaluation, while 88 smears (8%) were reported as unsatisfactory for evaluation. In the present study, main cause for unsatisfactory smears was found to be due to reduced squamous epithelial cellularity 70 smears (79.5%). 08 smears (9.1%) were unsatisfactory due to dense inflammation obscuring the squamous cellularity and 10 smears (11.4%) were reported as un-satisfactory due to both dense inflammations obscuring squamous cellularity and reduced squamous cellularity.

Of 999 satisfactory smears, 959(96%) were NILM (Negative for intra epithelial lesion/malignancy), 40 smears were positive for intraepithelial lesion/neoplasm. These 40 smears represented 4% (40/999) of the satisfactory smears and 3.6% (40 /1087) of the total smears examined (Table 1).

**Table 1: Distribution of total cases (n=1087).**

| Category | No. of cases |
|---|---|
| Unsatisfactory | 88(8.1%) |
| a) Reduced squamous cellularity | 70 |
| b) Inflammation | 08 |
| a and b Both factors | 10 |
| NILM | 959(88.2%) |
| Intra epithelial lesion/neoplasm | 40(3.7%) |
| Total | 1087 |

### *Non- neoplastic lesions including inflammatory smears and organisms*

Among the non-neoplastic lesions, the distribution of cases was as follows: 209 smears showed *bacterial vaginosis* (21.8%), *Candida* was reported in 77 smears (08%), both *bacterial vaginosis* and *Candida* was reported in 12 smears (1.2%), *Trichomonas vaginalis* was reported in 1 case, reactive cellular changes were reported in 193 smears (20.1%) and altered /mixed flora was reported in 112(11.7%) (Table 2) (Figure1).

All Intraepithelial lesions were categorized as: Atypical squamous cell of undetermined significance (ASCUS), low-grade squamous intraepithelial lesion (LSIL), Atypical squamous cell cannot exclude high grade

squamous intraepithelial lesion (ASC-H), high grade squamous intraepithelial lesion (HSIL), atypical glandular cells of undetermined significance (AGUS) and atypical glandular cells favoring neoplasm (AGC-FN). The malignant categories were squamous cell carcinoma (SCC), Adenocarcinoma and Malignancy, not otherwise specified.

Among the 40 neoplastic lesions, the distribution of smears was as follows: 16 of 40 (40%) were reported as ASCUS, 08 of 40 (20%) were reported as ASC-H, 04 of 40 (10%) reported as LSIL, 07 of 40 (17%) as HSIL, 01 (03%) as AGC-FN and 04 (10%) as SCC. No case was designated as LSIL-ASCH or positive for secondaries. (Table 3).

***ASC-US (Atypical squamous cell-Undetermined significance) and ASC-H (Atypical Squamous Cell-cannot rule out High Grade)***

Data wise 40% (16/40) of all epithelial abnormalities in the present study were reported as ASC-US. The overall ASC-US reporting rate was 1.6%. Out of 16 cases, 12 were lost to follow-up. Histology was available for 04 cases which turned out to be benign.

The nuclear abnormality which fell short of LSIL and more than reactive atypia were included in this category. The cytological features included nucleomegaly, high nuclear-cytoplasmic ratio (N:C ratio), mild nuclear hyperchromasia and multi-nucleation.

**Table 2: Distribution of non-neoplastic findings including inflammatory smears and organisms in NILM smears (n=959).**

| Category | No of cases |
|---|---|
| *Bacterial vaginosis* | 209(21.8%) |
| *Candida* | 77(08%) |
| Both | 12(1.2%) |
| *Trichomonas vaginalis* | 01(0.1%) |
| Altered/ mixed flora | 112(11.7%) |
| Reactive cellular changes | 193(20.1%) |

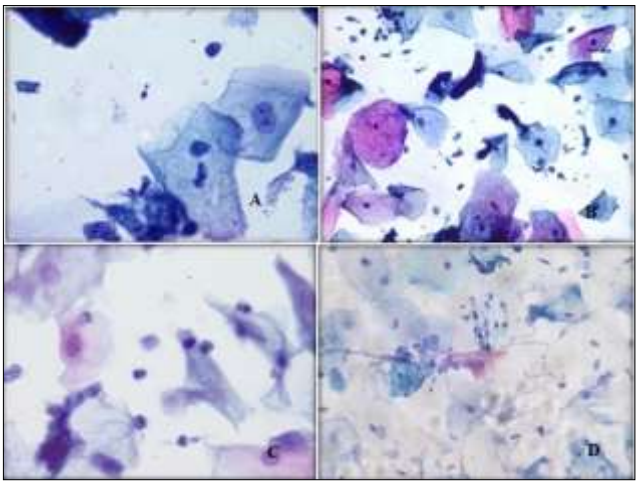

**Figure 1: A - *Candida* (yeast form). B - *Bacterial Vaginosis* with presence of clue cells. C - *Trichomonas vaginalis* (centre). D - *Candida* (pseudohyphae) pap stain liquid-based cytology 400x.**

**Table 3: Distribution of Intra-epithelial lesion/neoplasm with histological correlation(n=40).**

| Category | No. of cases | Lost to follow up | Follow up histology (benign ) | Follow up histology (intraepithelial lesion/malignancy) |
|---|---|---|---|---|
| ASCUS | 16 | 12 | 04 | - |
| ASC-H | 08 | 02 | 05 | 01 HSIL |
| LSIL | 04 | 01 | 03 | - |
| HSIL | 07 | 03 | - | 04 HSIL |
| SCC | 04 | - | - | 04(SCC) |
| AGC-FN | 01 | - | - | 01(Adeno-ca) |

Statistically 20% (08/40) of all epithelial abnormalities were reported as ASC-H. Histology was available for 6 cases. 01 case was reported as HSIL and remaining 05 cases were reported as chronic cervicitis. ASC-H refers to a group in which cytological changes are suggestive of HSIL.

Majority of cases showed groups of small cells with high N:C ratio with nuclear size only 2-3 times the size of neutrophil nuclei.

***LSIL (Low Grade Squamous Intraepithelial Lesion)***

Only 04 cases (10%) were reported as LSIL. Koilocytes were noted in all 04 cases. Main morphological features included intermediate sized squamous cells with nuclear enlargement, slight increase in N:C ratio, uniformly distributed, coarsely granular nuclear chromatin and slightly irregular nuclear membranes and peri-nuclear cavitation (Figure 2A). On histology, 03 cases were reported as chronic cervicitis. 01 case was lost to follow-up.

***HSIL (High Grade Squamous Intraepithelial Lesion) and Squamous cell carcinoma***

Furthermore 07 cases (17%) were reported as HSIL. Out of 07 cases, 04 cases on biopsy were reported as HSIL and 03 cases were lost to follow up. Main morphological parameters were cellular smears, hyperchromatic crowded cell groups with loss of polarity and associated with cellular abnormalities (Figure 2B). Background showed small dyskeratotic cells, which were conspicuous in LBC smears. 04 cases were reported as squamous cell carcinoma on LBC with histology confirming the same. Tumour diathesis (clinging) along with many dyskeratotic cells, tadpole cells, naked nuclei and moderate to dense inflammation were found in all cases (Figure 3 A, B).

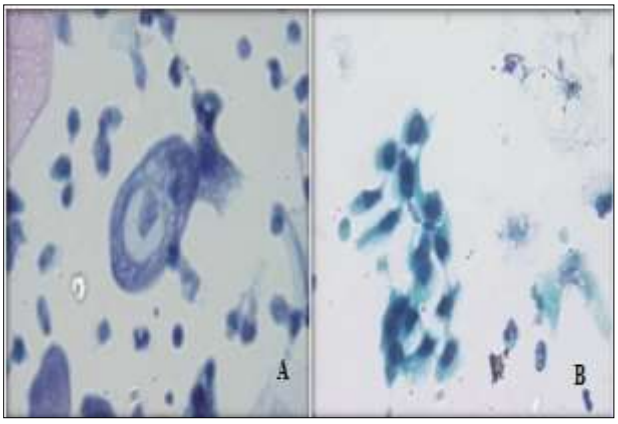


**Figure 2: A - Squamous cell showing koilocytic changes, perinuclear halo and cytoplasmic cavitation suggestive of low grade squamous intra epithelial lesion (LSIL), B - High grade squamous intra epithelial lesion (HSIL). Pap stain liquid-based cytology 400x.**

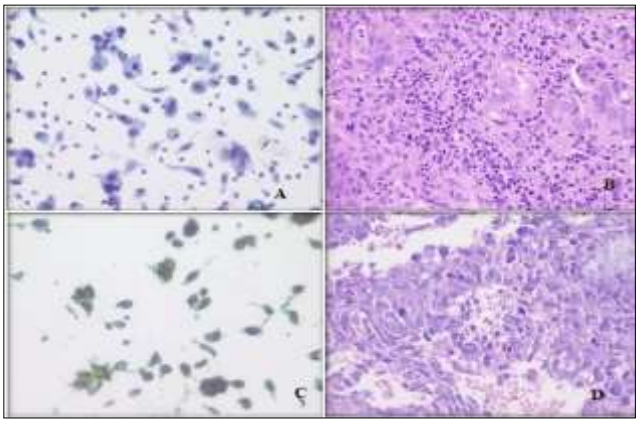


**Figure 3: A squamous cell carcinoma Pap stain liquid based cytology 400 x; B squamous cell carcinoma confirmed on histology, Hematoxylin and Eosin stain 400x ,C- Atypical glandular cell favoring neoplasm AGC-FN pap stain liquid based cytology 400x ;D Adenocarcinoma confirmed on histology, Hematoxylin and Eosin stain 400x.**

***Atypical glandular cells - favoring neoplasm***

Only 01 case was reported as AGC-FN, which on cervical biopsy was reported as endocervical adenocarcinoma (Figure 3 C, D).

## DISCUSSION

Success of cervical screening lies in its ability to reduce the incidence of and deaths from cervical cancer in a cost-effective manner.[5] Globally there have been two major advances in screening of cervical cancer which has led to lowering of death rate from cervical cancer. One of the earliest advances in cervical cancer screening test was the Pap test introduced in 1941 by George Papanicolaou as a cervical pathology screening test. Implementation of Pap testing was responsible for reducing the incidence of cervical cancer between 1955 and the mid-1980s. The second major advance in cervical cancer screening was Liquid-Based Cytology (LBC). Today, LBC accounts for more than 90% of the Pap tests performed in developed countries. This shift from conventional cytology to LBC has led to improvements in sample quality, reproducibility, sensitivity, and specificity, as well as the ability to perform reflex molecular testing for HPV high risk strains and others.[6,7]

In the present study, a high rate of reporting unsatisfactory cases (i.e. 8%) was noted. In a study conducted by Vikrant Bhar Singh et al, the rate of reporting unsatisfactory smears was 1.7%.[8] The high rate of reporting unsatisfactory smears in the present study was mainly due to inadequate/improper sampling. This implies the need of training health care professionals about adequate sampling in cervical LBC. However, the unsatisfactory smear rate was markedly lower when compared to Pap smears reported by conventional method in previous studies. In a study done by Kiran Rawat et al, in Western Rajasthan population, the rate of reporting unsatisfactory smears using conventional cytology was as high as 16.1%.[9]

In the present study, the rate of reporting non neoplastic findings including inflammatory smears and organisms was 62.9 % of satisfactory smears and 55.6 % of total smears. Kiran Rawat et al, reported the rate of inflammatory smears as (55.07%).[9] Among this sub-group were included reactive/ reparative cellular changes-193 smears (39%), *Bacterial vaginosis*- 209 smears (43%), *Candida*- 77 cases (16%), *Trichomonas vaginalis* -01 case and mixed flora in 112(11.7%) smears, 12 cases (2%) were reported as having both *Bacterial vaginosis* and *Candida*.

The inflammation associated with smears was graded as mild, moderate and severe. Though rate of reporting of inflammatory smears and organisms is comparable between LBC and conventional smears, infectious organisms such as *Candida pseudohyphae*, *Trichomonas vaginalis* and coccobacilli of *Gardenella vaginalis* were

visualized more easily on the LBC samples as compared to the conventional smears where the above findings are obscured by inflammation, mucus or blood.

The rate of reporting for intraepithelial lesion/ neoplasm was 04% of satisfactory smears and 3.6% of total smears. Various studies suggest prevalence rates of abnormal epithelial changes ranging from 1.4-7.8% in India.[9,10] Kiran Rawat et al, reported 3.2% cases as showing abnormal epithelial changes in conventional Pap smear study.[9] There were 24 cases were reported as ASC (atypical squamous cells (ASCUS+ASC-H) with positive histological concordance in only 1 case. On the other hand, in the present study, 11 cases were reported as HSIL and SCC with positive histological concordance for all cases for which the data was available. Hence, in the present study, the morphologic diagnosis of high-grade lesions (i.e. HSIL and SCC) on LBC showed stronger histological correlation than low grade lesions (ASC and LSIL). The ASCUS reporting rate in the present study was 1.6% and contributed to 40% of all intraepithelial abnormalities. It was the most common intraepithelial abnormality reported and the data is in concordance with previous studies.[7,11,12] The reporting of ASC is of prime importance especially in rural and underprivileged parts of India including Western Rajasthan as it contributes to increased sensitivity of detection of precancerous lesions that this screening test is designed to identify. The present study also highlights the neglected female health in Western Rajasthan, where a major section of women does not come for follow up visits despite being explained the severity and the fatality the disease can cause and the benefits of early detection of SIL(Squamous Intraepithelial Lesions) through this screening.

## CONCLUSION

To the best of our knowledge, the present study is the first and largest study of cervical liquid-based cytology in Western Rajasthan. In Western Rajasthan, where women health is still largely neglected, such screening tests aim to detect cervical cancer in early stages, reduce morbidity and mortality and have an impact on prognosis. Also, there is need of training of health care professionals about adequate sampling in cervical LBC. Implementation of cervical Liquid based cytology over conventional Pap smear reduces the rate of unsatisfactory smears and improves quality and reproducibility of smears. Standardized reporting formats further helps in reducing errors by bringing uniformity in reporting and leads to better clinician understanding. In addition, molecular testing for HPV high risk strains can be performed which can further help to detect at risk patients and reduce the burden of disease.

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