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

Neha Bagga¹, Poonam Elhence¹*, Meenakshi Rao¹, Aasma Nalwa¹, Sudeep Khera¹, Jyotsna Naresh Bharti¹, Pratibha Singh², Shashank Shekhar²

¹Department of Pathology, ²Department of Obstetrics and Gynaecology, AIIMS Jodhpur, Rajasthan, India

Received: 08 October 2019
Revised: 04 November 2019
Accepted: 04 November 2019

*Correspondence:
Dr. Poonam Elhence,
E-mail: poonamehence@gmail.com

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.¹³ 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.²⁴

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
- Hysterectomyed 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 unsat-
factory 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>
<thead>
<tr>
<th>Category</th>
<th>No. of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsatisfactory</td>
<td>88(8.1%)</td>
</tr>
<tr>
<td>a) Reduced squamous cellularity</td>
<td>70</td>
</tr>
<tr>
<td>b) Inflammation</td>
<td>08</td>
</tr>
<tr>
<td>a and b Both factors</td>
<td>10</td>
</tr>
<tr>
<td>NILM</td>
<td>959(88.2%)</td>
</tr>
<tr>
<td>Intra epithelial lesion/neoplasm</td>
<td>40(3.7%)</td>
</tr>
<tr>
<td>Total</td>
<td>1087</td>
</tr>
</tbody>
</table>

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-ASC 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).**

<table>
<thead>
<tr>
<th>Category</th>
<th>No of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial vaginosis</td>
<td>209(21.8%)</td>
</tr>
<tr>
<td>Candida</td>
<td>77(08%)</td>
</tr>
<tr>
<td>Both</td>
<td>12(1.2%)</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>01(0.1%)</td>
</tr>
<tr>
<td>Altered/ mixed flora</td>
<td>112(11.7%)</td>
</tr>
<tr>
<td>Reactive cellular changes</td>
<td>193(20.1%)</td>
</tr>
</tbody>
</table>

**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.**

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.

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

<table>
<thead>
<tr>
<th>Category</th>
<th>No. of cases</th>
<th>Lost to follow up</th>
<th>Follow up histology (benign)</th>
<th>Follow up histology (Intraepithelial lesion/malignancy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASCUS</td>
<td>16</td>
<td>12</td>
<td>04</td>
<td>-</td>
</tr>
<tr>
<td>ASC-H</td>
<td>08</td>
<td>02</td>
<td>05</td>
<td>01 HSIL</td>
</tr>
<tr>
<td>LSIL</td>
<td>04</td>
<td>01</td>
<td>03</td>
<td>-</td>
</tr>
<tr>
<td>HSIL</td>
<td>07</td>
<td>03</td>
<td>-</td>
<td>04 HSIL</td>
</tr>
<tr>
<td>SCC</td>
<td>04</td>
<td>-</td>
<td>-</td>
<td>04(SCC)</td>
</tr>
<tr>
<td>AGC-FN</td>
<td>01</td>
<td>-</td>
<td>-</td>
<td>01(Adeno-ca)</td>
</tr>
</tbody>
</table>

International Journal of Research in Medical Sciences | December 2019 | Vol 7 | Issue 12   Page 4575
**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).

**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. 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 pathalogy 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.

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%. 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%. 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%). Among this subgroup were included reactive/ reparative cellular changes-193 smears (39%), Bacterial vaginoses- 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 vaginoses 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.

REFERENCES

1. Chatterjee S, Chattopadhyay A, Samanta L, Panigrahi

P. HPV and cervical cancer epidemiology - Current

status of HPV vaccination in India. Asian Pac J Cancer


2. Sreedevi A, Javed R, Dinesh A. Epidemiology of
cervical cancer with special focus on India. Inter J

Women's Health. 2015;7:405-14.


V, Pramesh CS, et al. The growing burden of cancer in

India: epidemiology and social context. Lancet Oncol.


Complementary data on cervical cancer prevention.

ICO/IARC Information Centre on HPV and Cancer

(HPV InformationCentre). Human Papilloma virus and

Related Diseases in India. Summary Report 2019

Available at: www.hpvcentre.net/statistics/reports/IND.pdf.

5. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers


worldwide: sources, methods and major patterns in


6. Beerman H, Van Dorst EB, Kuenen-Boumeester V,

Hogendoorn PC. Superior performance of liquid-based

versus conventional cytology in a population-based
cervical cancer screening program. Gynecol Oncol.


7. Gibb RK, Martens MG. The Impact of Liquid-Based

Cytology in Decreasing the Incidence of Cervical


V, Rajwanshi A. Liquid-based cytology versus

conventional cytology for evaluation of cervical Pap

smears: experience from the first 1000 split samples.


9. Rawat K, Rawat N, Mathur N, Mathur M, Chauhan N,

Tinna R, et al. A study of cytological pattern of
cervical papanicolaou smears in western Rajasthan,


2017;5(9):3186-90.

10. Misra JS, Srivastava S, Singh U, Srivastava AN. Risk-

factors and strategies for control of carcinoma cervix in

India: hospital based cytological screening experience of


11. Mulay K, Swain M, Patra S, Gowrishankar S. A

comparative study of cervical smears in an urban

Hospital in India and a population-based screening

program in Mauritius. Indian J Pathol Microbiol.


B, Singhal M, et al. Meeting report,“First Indian

national conference on cervical cancer management-

expert recommendations and identification of barriers
to implementation. 2018;1-12.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the

Institutional Ethics Committee

Cite this article as: Bagga N, Elhence P, Rao M,


study of cervical lesions diagnosed by liquid based
cytology in western Rajasthan, India population. Int J