

# Conventional Pap Smear and Liquid Based Cytology for Cervical Cancer Screening – A Comparative Study

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## Abstract

The study was undertaken to evaluate liquid based cytology (Pap spin) and to compare the sensitivity of Pap spin with conventional Pap smear. Pap smears were taken from 160 patients with gynaecologic complaints with Ayre's spatula and endocervical cytobrush and slides prepared. The residual material on both the spatula and cytobrush were rinsed in Pap spin collection fluid and centrifuged. Direct smears were prepared from the cell button, fixed and stained by Papanicolaou stain. Colposcopy was performed in patients with abnormal smears and biopsy was performed in suspected malignant or dysplastic cases. LSIL and HSIL was diagnosed in 27 (64.4%) cases, mostly between 21-40 years, Commonest presenting complaint was discharge per vaginum seen in 68 (42.5%) cases. Cytological abnormality was found in 42 cases (26.2%) by Pap spin method, whereas conventional Pap smear detected abnormality in only 24 cases (15%). 133 cases (83.1%) were satisfactory for evaluation on Pap spin and 51 cases (31.9%) on conventional Pap smear. The commonest atypical finding on colposcopy was acetowhite area in 14 cases (31.1%). Sensitivity of Pap spin and conventional Pap smear was 97.6% and 53.7% respectively and specificity was 50% in each. Pap spin is strongly advocated in the best interest of public health as it improves the sample quality and reduces the likelihood of false negative results.

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**Key Words :** Liquid based cytology, conventional Pap smear, cytology.

## Introduction

Cervical cytology was introduced by George Papanicolaou into clinical practice in 1940.<sup>1</sup> In 1945, the Papanicolaou smear received the endorsement of the American cancer society as an effective method for the prevention of cervical cancer. Center of cytology in Vancouver, British Columbia published data which confirmed that cytologic screening leads to a reduction in the rate of invasive cancer of the uterine cervix.

Park et al<sup>2</sup> established that the sensitivity of the conventional Pap smears for the detection of cervical cancer precursors was less than 50%. Several limitations of conventional smear were identified including inadequate transfer of cells to slide,<sup>3</sup> in homogenous distribution of abnormal cells, presence of obscuring blood, inflammation or thick areas of

overlapping epithelial cells.<sup>4</sup>

Liquid based, thin layer technology was developed to address the limitation of Pap smear. More than 5,00,000 subjects have been studied with a preponderance of data indicating a significant benefit of liquid-based, thin layer technology in the detection of cervical cancer precursor lesions and in the improvement of specimen adequacy.

The present study was undertaken to evaluate a liquid based cytology technique (Pap spin) and to compare the sensitivity of Pap spin with conventional Pap smear.

## Materials and Methods

The present study was conducted on the patients, attending the inpatients and outpatients departments

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Patients were randomly selected on the basis of complaints like bleeding per vaginum, irregular menses, pain in lower abdomen, post coital bleeding or any abnormal findings on per speculum examination. After a detailed history and thorough clinical examination, Pap smears were taken from the cervix with an Ayre's spatula and endocervical cytobrush and slides prepared. Then residual material on both the spatula and cytobrush were rinsed in 10-15 ml of Pap spin collection fluid—Liqui-PREP™ preservative solution (LGM International, Inc.; Ft. Lauderdale, Florida, USA). Pap spin collection fluid along with the collected material was transferred to test tubes and spun in Shandon cytospin. After centrifugation at 1500 rpm for 10 minutes, supernatant was decanted and direct smear was prepared from the cell button; fixed in 95% ethyl alcohol for 20-30 minutes and stained by Papanicolaou stain. Colposcopy was performed by Olympus® colposcope in patients with abnormal smears and colposcopic guided biopsy was performed in suspected malignant or dysplastic cases.

### Observations

The present study was carried out on 160 patients attending the inpatients and outpatient Department of Gynaecology and Pathology of JN Medical College Hospital, AMU, Aligarh. After a detailed clinical history

and thorough examination; per speculum examination was done and specimen collected for preparation of both conventional Pap smear and liquid-based cytology (pap spin). Colposcopy and biopsy was carried out in 45 cases with abnormal cytology either on Pap spin or conventional Pap smear.

77 (48.1%) cases studied belonged to fourth decade of life, followed by 50 (31.2%) cases in the third decade. The minimum age of patient screened was 21 years and maximum was 63 years. Low grade squamous intraepithelial lesion (LSIL) and high grade squamous intraepithelial lesion (HSIL) were found in 27 (64.4%) cases in patient's aged between 21-40 years (Table 1).

Out of 160 cases, 154 (96.2%) started sexual activity before 25 years of age. 23 cases (71.9) of LSIL and 5 cases (71.4%) of HSIL had onset of sexual activity before 20 years of age. 61 (38.1%) cases had more than 5 children. No cytological abnormality was found in women who had started sexual activity after 25 years of age.

Majority of the cases, 104 (65%) belonged to class II and III (modified Prasad's Classification<sup>5</sup>). Out of the 45 cases with dysplasia / carcinoma, 24 cases (53.3%) belonged to class III (Table 2).

Most common presenting complaint was discharge per vaginum in 68 (42.5%) cases, followed by pain lower abdomen in 44 (27.5%) cases and menstrual irregularity in 38 cases (23.8%). Postmenopausal

**Table 1: Age-wise case distribution**

| Age (yrs) | Total | No. of cases<br>Normal | Abnormal | Low grade squamous<br>intraepithelial<br>lesion (LSIL) | High grade squamous<br>intraepithelial<br>lesion (HSIL) | Invasive<br>carcinoma |
|-----------|-------|------------------------|----------|--|---|-----------------------|
| 21-30     | 50    | 43                     | 7        | 5  | 2   | -                     |
| 31-40     | 77    | 55                     | 22       | 16   | 4   | 2                     |
| 41-50     | 19    | 11                     | 8        | 6  | 1   | 1                     |
| 51-60     | 10    | 6                      | 4        | 3  | -   | 1                     |
| 61-63     | 4     | -                      | 4        | 2  | -   | 2                     |
| Total     | 160   | 115                    | 45       | 32   | 7   | 6                     |

**Table 2 : Case distribution according to socio-economic status (modified Prasad's classification<sup>5</sup>)**

| Class | Total no. of cases | Normal cases | Abnormal cases | LSIL | HSIL | Invasive carcinoma |
|-------|--------------------|--------------|----------------|------|------|--------------------|
| I     | 36                 | 35           | 01             | 01   | -    | -                  |
| II    | 47                 | 40           | 07             | 07   | -    | -                  |
| III   | 57                 | 33           | 24             | 21   | 01   | 02                 |
| IV    | 17                 | 05           | 12             | 03   | 05   | 04                 |
| V     | 03                 | 02           | 01             | -    | 01   | -                  |
| Total | 160                | 115          | 45             | 32   | 07   | 06                 |

bleeding was the presenting complaint in 50% cases of invasive carcinoma.

Out of 160 cases, cytological abnormality was found in 42 cases (26.2%) by Pap spin method, whereas conventional Pap smear detected abnormality in only 24 cases (15%) (Table 3).

133 (83.1%) cases were satisfactory for evaluation on Pap spin whereas 51 (31.9%) cases were satisfactory on conventional Pap smear. 6 cases (3.7%) were unsatisfactory for evaluation on Pap spin and 8 cases (5%) on conventional Pap smear. There were only 21 cases (13.2%) which were satisfactory for evaluation but limited by factor like air drying artifact, obscuring blood and inflammation, cytolysis or absent endocervical component on Pap spin whereas 101 (63.1%) cases in the same category on conventional Pap smear. The most common cause of unsatisfactory smear on Pap spin was scant cellularity in 3 cases (1.9%) and on conventional Pap smear, thick smear was the commonest cause in similar percentage of cases.

Infections agents were detected in 14 (8.7%) cases on Pap spin and in 5 (3.1%) cases on conventional Pap smear. Candida was the commonest infectious agent in 7 (4.3%) cases, followed by *Trichomonas vaginalis* in 4 cases (Fig.1), out of which 6 cases

(85.8%) were detected on Pap spin smears.

A comparative study of Pap spin and histopathological findings was performed. Out of the 29 cases diagnosed as LSIL on Pap spin (Fig.2), 2 (6.9%) case each had normal histology and moderate dysplasia and 26 (89.7%) cases had mild dysplastic changes on histopathology. Out of the 7 cases of HSIL (Fig.3) 1 (14.3%) had mild dysplasia and 6 (85.7%) cases had moderate dysplasia and all the 6 cases of carcinoma on Pap spin revealed squamous cell carcinoma on histopathology.

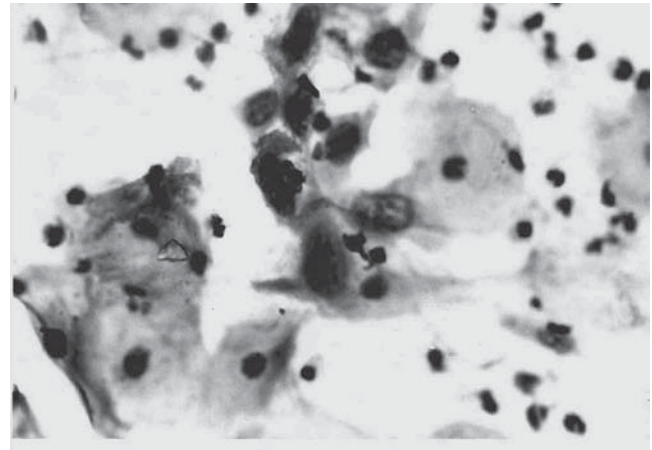


Fig. 2 : Pap Spin smear showing evenly dispersed cells with preserved inflammatory cells and LSIL (Pap, x 400).

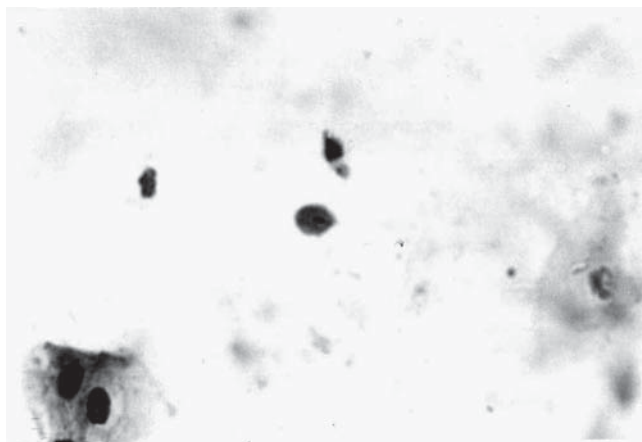


Fig. 1 : Pap Spin smear showing grayish blue pear shaped body with a single ovoid nucleus of trichomonas vaginalis (Pap, x 400).

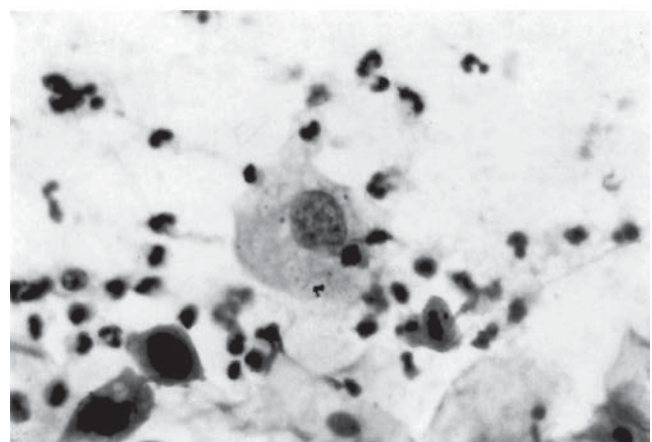


Fig. 3 : Pap Spin smear showing a group of atypical squamous cells diagnosed as HSIL (Pap x 400).

**Table 3 : Case distribution of Pap spin and conventional Pap smear**

| Category  | Pap spin     |            | Conventional pap smear |            | p value |
|-----------|--------------|------------|------------------------|------------|---------|
|           | No. of cases | Percentage | No. of cases           | Percentage |         |
| Normal    | 118          | 73.8       | 136                    | 85.0       | -       |
| LSIL      | 29           | 18.2       | 17                     | 10.6       | < 0.05  |
| HSIL      | 07           | 4.3        | 01                     | 0.6        | < 0.05  |
| Carcinoma | 06           | 3.7        | 06                     | 3.7        | -       |
| Total     | 160          | 100        | 160                    | 100        |         |

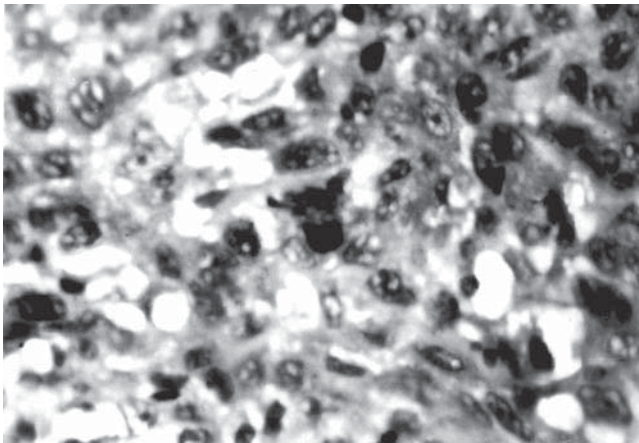


Fig. 4 : Histological section showing large cell non keratinizing squamous cell carcinoma of cervix (H&E, x 400).

A similar comparative study between findings on conventional Pap smear and histopathology revealed that out of 17 cases diagnosed as LSIL on conventional Pap smear, 2 each (11.8%) had normal histology and moderate dysplasia whereas 13 cases (76.5%) had mild dysplasia on histopathological study. A single case of HSIL on Pap smear was diagnosed as moderate dysplasia on histopathology, whereas all the 6 cases of carcinoma on Pap smear were diagnosed as squamous cell carcinoma (large cell non keratinizing type) on histopathology (Fig.4).

On colposcopic evaluation of patients, the commonest atypical finding was acetowhite area in 14 (31.1%) cases (Fig.5), followed by punctuations in 7 (15.5%) cases, abnormal blood vessels in 5 (11.1%) and mosaics in 3 (6.7%) cases. Out of the 14 cases with acetowhite area, 3 (21.4%) cases were diagnosed as mild dysplasia, 5 (35.7%) as moderate dysplasia and 6 (42.8%) cases as carcinoma on histopathology (Table 4).

In our study, sensitivity and specificity of Pap spin was 97.6% and 50.0% respectively and of conventional Pap smear 53.7% and 50% respectively.

## Discussion

The Pap smear has been utilized for cervical cancer

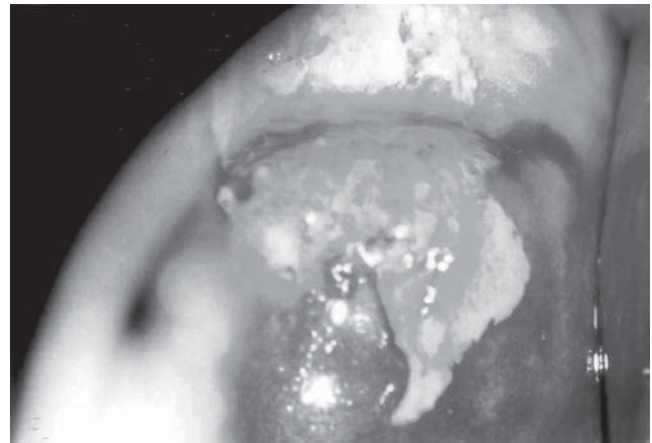


Fig. 5 : Colposcopy examination showing acetowhite areas.

screening for more than 50 years. Despite being credited with a 70% reduction in mortality for cervical cancer, the false negative rate is still a cause for concern. It is widely acknowledged that two third of the overall false negative rate can be attributed to sampling errors. Liquid based cytology has been developed to address the sampling problems of conventional Pap smear. The present work was done to evaluate the liquid based cytology and to compare the sensitivity of the same with conventional Pap smear.

There were 77(48.1%) cases in the fourth decade of life, Cases of LSIL were mostly found in the fourth decade; a finding similar to that reported by Richart.<sup>6</sup> Our study revealed cases of invasive cancer at a younger age of early fourth decade; a finding in contrast to studies by Parker et al<sup>7</sup> who reported carcinomatous cases beyond the age of 70 years. Our observations may be due to a common practice of early marriage and sexual activity in our country.

Majority of the cases of dysplasia and carcinoma on cervical cytology had parity of five or more; a finding concordant to the studies of Shankarnarayana et al.<sup>8</sup> Parker et al<sup>9</sup> also commented that seven or more full term pregnancies had a four fold increase in the risk of developing squamous cell carcinoma of the cervix.

**Table 4 : Comparative study of colposcopic findings and histopathology**

| Colposcopic findings   | No. of cases | Histopathology |                    |                  |           |
|------------------------|--------------|----------------|--------------------|------------------|-----------|
|                        |              | Mild dysplasia | Moderate dysplasia | Severe dysplasia | Carcinoma |
| Acetowhite area        | 14           | 3              | 5                  | -                | 6         |
| Punctuation            | 04           | -              | 3                  | -                | 4         |
| Mosaic                 | 03           | -              | 1                  | -                | 2         |
| Abnormal blood vessels | 05           | -              | 1                  | -                | 4         |
| Total                  | 29           | 03             | 10                 | -                | 16        |

Our study revealed majority of dysplastic (71.8%) and carcinomatous cases (71.04%) had onset of sexual activity in the late second decade as has been reported by workers like Rotkin.<sup>10</sup> He postulated that carcinoma of cervix is a disease transmitted from male to female during intercourse, with a higher probability of occurrence with early age at first coitus.

In our study out of the 45 cases with cytological abnormality, 37 (82.2%) cases belonged to class III or lower. This finding is similar to that noted by Christopherson and Parker,<sup>11</sup> who noted a high incidence of disease in women of low socio-economic class with younger age of marriage and child bearing.

The most common presenting complaint in our study was discharge per vaginum (42.5%). Kenneth and Yao<sup>12</sup> have emphasized the significance of vaginal discharge and its association with neoplastic changes in the cervix. All the patients with post coital bleeding had moderate dysplasia (66.7%) and carcinoma (33.3%). These results are consistent with the known association of cervical neoplasia with post coital bleeding.<sup>13</sup>

In the present study detection of LSIL increased from 10.6% to 18.1% ( $p < 0.05$ ) with Pap spin than with conventional Pap and detection of HSIL increased from 0.6% to 4.3% ( $p < 0.05$ ). Hutchinson et al<sup>3</sup> showed that fewer than 20% of cells collected by Pap smears were transferred on to the slide and thus explained the high prevalence of true false-negative rate. By rinsing the sampling device into a liquid fixture helps the entire sample to be captured into the vial. They reported a higher percentage of cases of LSIL on liquid based cytology (10.6%) than conventional Pap smear (9.0%). Diaz-Rosario and Kabawat<sup>14</sup> reported increased detection of premalignant precursors on liquid-based cytology as compared to conventional smear. They reported an increased percentage of cases of LSIL from 1.6% to 2.7% and of HSIL increased from 0.3% to 0.5%.

In our study satisfactory smears on Pap spin were 83.1% as compared to 31.9% on conventional Pap smears. Quite similarly Weintraub and Morabia<sup>15</sup> have reported an increased number of satisfactory cases (72.2% to 92%) on liquid based cytology than conventional smears. All drying artefact and cytolysis is almost absent or minimal with liquid based cytology because of immersion of cells into the liquid fixative and specimen adequacy was greatly improved due to absence of limiting factors like blood, mucus and inflammatory cells. Only conventional smears were unsatisfactory due to thick smear, which was not a

problem with liquid based cytology due to even distribution of cells.

The microscopic details of infectious agents were enhanced on Pap spin with candida, coccobacilli and trichomonas being readily detected.

In our study sensitivity and specificity of Pap spin was 97.6% and 50% respectively and of conventional Pap smear 53.7% and 50% respectively. Very similarly Bolick et al<sup>4</sup> reported sensitivity and specificity of liquid based cytology as 95.2% and 58% respectively whereas on conventional Pap smear, the same was 85% and 36% respectively.

In our colposcopic study; acetowhite area was seen in 14 out of 45 cases (31.1%), punctuations in 7 (15.5%) cases, mosaic in 3 (6.7%) and abnormal blood vessels in 5 (11.1%) cases. Our findings are consistent with the observations of Kenneth and Yao,<sup>12</sup> who had observed acetowhite areas in 30%, punctuations in 5.7% and mosaic pattern in 3.5% cases. Acetowhite area was seen in 100% cases of carcinoma and 20.5% cases of dysplasia in our study a finding similar to Campion et al,<sup>16</sup> who reported 100% of incidence of carcinoma in acetowhite areas.

## Conclusion

Liquid based cytology is strongly advocated in the best interest of public health, by improving the quality of the sample and reducing the likelihood of false negative cytology results. Thus it will significantly improve early detection and treatment of cervical lesions.

## References

1. Papanicolaou GN. Introduction of Pap smear in early detection of cervical malignancies. *Am J Clin Path* 1940; 19: 301-8.
2. Park IA, Lee SN, Chaw SW, Kim JW. Comparing the accuracy of thin prep pap tests and conventional pap smears on the basis of the histologic diagnosis - a clinical study. *Acta Cytol* 2001; 45: 525-31.
3. Hutchinson ML, Agarwal P, Denant T, Cibas E. A new look at cervical cytology: thin prep multicenter trial results. *Acta Cytol* 1992; 36: 499-54.
4. Bolick DR, Hellman DT. Laboratory implementation and efficacy assessment of the thin prep cervical cancer screening system. *Acta Cytol* 1998, 42: 209-13.
5. Rao TB. Methods of social classification. Rao's sociology in medicine, 1st ed. Guntur: Sree Graphics; 2002.
6. Richard RM. Natural history of cervical intraepithelial neoplasia. *Clin Obstet Gynaecol* 1968; 10: 748-50.
7. Parker SL, Tong T, Bolden S, Wing PA. Cancer statistics 1996; *CA Cancer J Clin* 1996; 46: 5-28.
8. Shakarnarayana R, Nene BM, Dinshaw K, Raj Kumar. Early detection of cervical cancer with visual impaction methods: a summary of completed and ongoing studies in India. *Shawad*

- Publica de Mexico* 2003; 45: 291-301.
9. Parker DM, Pisani P, Ferlay J. Cancer incidence, mortality and prevalence worldwide. Ver. 1, IARC, Cancer No.5, Lyon Press, 2001.
  10. Rotkin. Epidemiology on cancer of the cervix. Sexual characteristics of cervical cancer population. *Am J Public Health* 1973; 57: 815-29.
  11. Christopherson WM and Parker JE. Poor socioeconomic condition and its association with carcinoma cervix. *Cancer* 1960; 13: 711-5.
  12. Kenneth DH, Yao S Fu. Cervical and vaginal cancer, Novak's Textbook of Obstetric and Gynaecology, 13th ed. Baltimore: WB Saunders Co; 2002.p.471-93.
  13. Robert ME, Fu YS. Squamous cell carcinoma of the uterine cervix - a review with emphasis on prognostic factors. *Semin Diagn Pathol* 1990; 7: 173-89.
  14. Diaz Rosario LA, Kabawat SE. Performance of a fluid based thin layer Papanicolaou smear method in the clinical setting of an independent lab and outpatient screening of population in New England. *Arch Path Lab Med* 1999, 123: 817-21.
  15. Wintraub J, Morabia A. Efficacy of a liquid based-thin layer method of cervical cancer screening in a population with a low incidence of cervical cancer. *Diagn Cytopathol* 2000; 22: 52-9.
  16. Campion MJ, McCance DJ, Gizick J, Singer A. Progressive potential of mild cervical atypia: prospective cytological and colposcopic study, *Lancet* 1986; 2: 237-40.