



Comparative study between liquid-based cytology & conventional Pap smear for cytological follow up of treated patients of cancer cervix

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Background & objectives: Cancer cervix is one of the most common forms of genital malignancy among Indian women. Recurrence is seen in a significant number of cases. The conventional cervical smear technique has inherent problems and screening and interpretation errors. This study was undertaken to assess the efficacy of liquid-based cytology (LBC) as a method for cytological follow up and detection of recurrence in treated cases of cancer cervix and to compare it with conventional Pap smear technique to find the best screening method for detection of recurrence in these patients.

Methods: This cross-sectional study was conducted over a period of one year. Patients attending Gynecology and Radiotherapy outpatient departments for follow up after treatment of cancer cervix were included. Pap smear and LBC were taken in all cases. Colposcopy and biopsy were done for those having epithelial cell abnormality in cytology report. Colposcopy and biopsy were taken as gold standard for diagnosis of cancer cervix recurrence.

Results: Ninety four treated patients of carcinoma cervix were studied. The diagnostic accuracy for detection of recurrence of conventional Pap smear was 79.16 per cent, and that of LBC was 97.6 per cent. The difference between the two methods was significant ($P < 0.001$).

Interpretation & conclusions: Our findings showed that LBC performed better than the conventional method of cytology to detect recurrence of squamous cell carcinoma. Its sensitivity, specificity as well as accuracy were much higher than conventional method. LBC can be a better method of cytological follow up of post-treated patients of cancer cervix.

Key words Cervical cancer - cytological follow up - liquid-based cytology - Pap smear - recurrence - treated cancer cervix

Cervical cancer worldwide is second only to breast cancer in incidence and approximately three-fourths of cases occur in the developing countries¹. Among Indian women, it is the most common form of genital malignancy². Recurrence is seen in a significant number of cases despite advances in universal screening,

early detection, surgical treatment and radiotherapy. Recurrence rates of 1.5 per cent for early small tumours and 20-40 per cent for more advanced tumours have been reported³. The National Comprehensive Cancer Network Guidelines⁴ for surveillance after cervical cancer treatment recommend conventional Pap testing

every three months for first two years, every six months for three years and then annually afterwards. The conventional cervical smear technique has been used for screening of cervical cancer since 1940s but has high false-negative rates due to sampling errors, presence of obscuring materials, screening and interpretation errors^{5,6}. Morphologic changes due to radiotherapy make it extremely difficult to interpret conventional Pap test result⁷. Liquid-based cytology (LBC) has advantages of fewer unsatisfactory smears, faster and more efficient method, more accurate interpretation, less obscuring materials such as blood, mucous, inflammatory cells in smears and the use of residual cell suspension for testing human papillomavirus (HPV) DNA and immunohistochemistry⁸. Studies show that there is high rate of satisfactory samples in post-radiotherapy patients with use of liquid-based cytology⁹. Vaginal vault cytology conducted using cytobrush was found to be more efficient due to adequate sample collection¹⁰.

In view of the emerging need of finding an effective tool for detecting recurrence of cervical cancer, this study was planned to assess the efficacy of LBC as a method for cytological follow up and detection of recurrence in treated cases of cancer cervix and compared with Pap smear method to find the best screening method for detection of recurrence in these patients.

Material & Methods

This cross-sectional observational study was conducted over a period of one year (January 2012-January 2013) in the department of Obstetrics and Gynecology, King George's Medical University, Lucknow, India. Patients of cancer cervix attending outpatient department of Obstetrics and Gynaecology and Radiotherapy department were selected for the study. An informed written consent explaining the procedure, its utility and safety were taken. The study protocol was approved by the institutional ethics committee. Inclusion criteria were patients of carcinoma cervix who had received treatment in the form of surgery and/or radiotherapy and/or chemoradiation and had completed their treatment as per the institutional protocol, were willing to take part in the study were included. Patient's with incomplete therapy and not willing to take part in the study were excluded. All patients underwent clinical assessment including age, parity, marital status, literacy status, age at menopause, chief complaints before diagnosis, mode of treatment, duration since treatment at the time

of recruitment, complaints if any after completion of treatment. Family history of genital and breast cancer (if any) was recorded. All cases underwent general examination (blood pressure, PR, temperature, pallor, oedema, icterus, etc.) followed by systemic examination (central nervous system, cardiovascular system, respiratory system and abdominal examination) including gynaecological examination. Pap smear was taken in all cases. Pap smear was taken by scraping cells from squamocolumnar junction with the help of Ayre's spatula. The scraping was evenly spread onto the glass slide which was immediately fixed in 95 per cent ethyl alcohol. The smears were stained by Papanicolaou stain and examined by cytopathologist. Pap smear reporting was done according to Bethesda classification¹¹. Cervex-Brush was used for sample collection in liquid-based cytology. Further processing was done according to BD SurePath™ method (BD, USA). Colposcopy was done in patients having lesions suspicious of cancer cervix or in patients having abnormal cytology. Biopsy was done in patients with abnormal colposcopy (combined Reid Colposcopic Index of three or more). Colposcopy and biopsy were taken as gold standard for diagnosis of cancer cervix recurrence.

Statistical analysis: Statistical analysis was done using SPSS version 15.0 (SPSS, IBM Corp., Armonk, NY, USA). With confidence of 95 per cent and power of 80 per cent and odds ratio (OR) 0.2, the sample size was calculated to be 85.9, hence 94 patients were selected (random sample).

Results

Ninety four patients of carcinoma cervix, who fulfilled the inclusion criteria, were studied. Mean age of patients was 49.5±10.9 years. Majority of cases were multiparous and illiterate (66%). Patient's detail regarding pre-treatment clinical staging was available for 67 patients only. Majority of patients were in Stage II (n=39, 58.2%), followed by 26.9 per cent (n=18) in Stage III. In 27 cases, record of clinical stage could not be retrieved. Squamous cell carcinoma (n=89, 94.7%) was the most common histopathology. Chemoradiation was the most common treatment modality (n=31, 33.0%), followed by 24.5 per cent patients (n=23) who received surgery and radiotherapy both, 20.2 per cent (n=19) had surgery alone and 17 per cent (n=16) had radiotherapy alone. Only 5.3 per cent patients (n=5) received all the three modalities *i.e.*, surgery, radiotherapy and chemotherapy. Maximum number

of patients (n=57, 60.6%) were followed up for two years after their primary treatment. About 79.8 per cent patients (n=76) who came for follow up were symptomatic, and the most common complaint was pain in lower back/abdomen (n=39, 40.4%) followed by discharge per vaginum (n=16, 17%). All patients had cervical/vaginal vault cytology by Pap smear and LBC.

Table I. Comparison of cytological findings by conventional Pap smear and liquid-based cytology (n=94)

Outcome	Conventional Pap (n=94) n (%)	Liquid based cytology (n=94) n (%)
Inadequate	20 (21.28)	7 (7.45)**
Negative for malignancy	26 (27.66)	61 (64.89)***
Inflammation	5 (5.31)	12 (12.7)
Infection	9 (9.57)	18 (19.14)
Epithelial cell abnormalities (squamous)	48 (51.06)	26 (27.66)***
ASCUS	0	0
LSIL	34 (36.17)	12 (12.77)***
HSIL	14 (14.89)	7 (7.45)
SCC	0	7 (7.45)**
Epithelial cell abnormalities-AGCUS	0	0

P **<0.01, ***<0.001 compared to conventional Pap. ASCUS, atypical squamous cell of undetermined significance; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; AGCUS, atypical glandular cells of undetermined significance

Table I shows the comparison of cytological findings of conventional Pap smear and LBC. Significant number of negative for malignancy cells were reported by LBC compared to conventional Pap ($P<0.001$). The overall sensitivity of conventional Pap smear was 37.3 per cent, specificity 84.3 per cent, positive predictive value 23 per cent and negative predictive value was 91.5 per cent while for LBC the overall sensitivity of LBC reports was 100 per cent and specificity was 97.29 per cent. Positive predictive value of LBC was 83.33 per cent while negative predictive value was 100 per cent. Table II shows the diagnostic accuracy of LBC versus Pap smear.

Discussion

Recurrence of cervical cancer is a difficult clinical problem and carries a poor prognosis; hence, early detection is the key for better survival of the patients³. The main goal of post-treatment surveillance is early detection of recurrence to improve the survival^{3,12-14}. In the present study, most common histopathological type of cancer found was squamous cell carcinoma (94.7%) and only 5.3 per cent cases were adenocarcinoma. Similar results were reported in a study carried out to observe the patterns of recurrence and post-treatment surveillance of cancer cervix patients in which squamous cell carcinoma accounted for 80 per cent of cervical cancers, while adenocarcinoma for 15 per cent cases¹⁵.

In our study, conventional Pap smear reports were inadequate in 21.28 per cent individuals, while there were only 7.45 per cent inadequate reports in liquid-based cytology. This difference between the

Table II. Diagnostic accuracy of conventional Pap smear (n=72) and liquid-based cytology (n=84)

Cytology method	Biopsy and colposcopy		Diagnostic accuracy (%)
	Normal, CIN-I and CIN-II/low grade, intermediate grade	CIN-III and malignancy/high-grade lesion	
Conventional Pap smear			
Negative + LSIL	54	5	79.16
HSIL + SCC	10	3	
Total	64	8	
LBC			
Negative + LSIL (n=72)	72	0	97.6
HSIL + SCC (n=12)	2	10	
Total	74	10	

Diagnostic accuracy of LBC was 97.6 per cent, and that of conventional Pap smear was 79.16 per cent. CIN, cervical intraepithelial neoplasia; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion; SCC, squamous cell carcinoma; LBC, liquid-based cytology

two techniques was found to be significant ($P=0.007$). This finding was much lower to that found in a study in which 64.1 per cent samples were inadequate by conventional Pap smear, while in case of LBC it was 38.7 per cent, the difference was significant ($P=0.008$)¹⁰. Various other studies found decrease in the percentage of unsatisfactory samples after conversion from conventional Pap smear to LBC^{5,16}. This difference may be because all drying artifacts as cytolysis is minimal with LBC because of immersion of cells into the liquid fixative. The number of cells also increases with LBC as sampling is done by cytobrush. Conventional smears are inadequate due to thick smear, which is not a problem of LBC due to even distribution of cells in the liquid medium.

In our study, total epithelial cell abnormalities were detected in 51.06 per cent by conventional Pap smear, while 27.66 per cent were detected by LBC. LBC reported 7.45 per cent cases as squamous cell carcinoma while no cases were reported by conventional Pap smear cytology ($P<0.01$). A study aimed to analyze the differences between the conventional and liquid-based cytology, found that LBC resulted in the diagnosis of some entities missed in conventional cytology [atypical squamous cells of undetermined significance (ASC-US), ASC-US associated to AGC-not otherwise specified, high-grade squamous intraepithelial lesion (HSIL) associated to AIS]¹⁷. LBC also provided the identification of higher number of cases of associated lesions. A study done to compare conventional and the LBC found that LBC had a better performance to diagnose atypical cells and the cyto-histological concordance was higher than in conventional cytology¹⁸.

Recurrence of squamous cell carcinoma occurred in five cases, all of whom were picked up by LBC (as HSIL or SCC), while none by conventional Pap smear. Of the five biopsy-proven, three were detected as squamous cell carcinoma and two as HSIL by LBC, while by conventional Pap smear cytology two were reported as inadequate, one as negative for malignancy and two as LSIL. McKenzie *et al*¹⁹ found that out of 44 recurrent cases only 24 (54.54%) were reported as malignant by conventional Pap smear and in another study²⁰, of the 147 cases of recurrence, only 12 (8.1%) were detected by conventional Pap smear. A study carried out to detect recurrence in post-radiotherapy patients of cancer cervix found sensitivity of conventional Pap smear 16.66 per cent, specificity 96.15 per cent, positive predictive value 83.33 per

cent, negative predictive value 42.37 per cent and diagnostic accuracy of the test was 46.15 per cent⁷. Studies showed that the sensitivity of conventional cytology was more in general population, and conventional Pap smear was 53.7 per cent sensitive and 50 per cent specific for screening purpose^{21,22}. In a study on post-radiotherapy patients, the sensitivity of LBC was 50 per cent, specificity and positive predictive value were 100 per cent⁹. Before treatment, cervical cells are non-irradiated and can be easily picked up by conventional method. While in case of post-treated patients, cervical stenosis, narrowing of vagina, dryness and radiotherapy-induced changes cause difficulty in sampling as well as interpretation by conventional method proving LBC to be a better choice.

In conclusion, the present results showed that the LBC performed better than the conventional method of cytology to detect recurrence of squamous cell carcinoma. Its sensitivity, specificity as well as accuracy were much higher than the conventional method. LBC can be better method of cytological follow up of post-treated patients of cancer cervix apart from a reduction in inadequate smears.

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Conflicts of Interest: None.

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