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Research Article

Does Liquid-Based Technology Improve Detection of Cervical Epithelial Abnormalities and Worth in Low Resource Setting?

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Abstract

Introduction: Cervical cancer is the most frequent cancer among Nepalese women.

Aims: This study was undertaken to assess the strength of liquid-based cytology (LBC) and conventional Pap smear (CPS) in detecting cervical dysplasia/cancer, and assess feasibility of LBC in our setting.

Methods: It was a cross-sectional study. Samples were collected from 312 patients for CPS and LBC by split sampling technique. Smears were interpreted according to the Bethesda System. The results between two methods were compared and analyzed statistically by applying Chi-square and t-tests.

Results: There was no significant difference in adequacy rates, representativeness, detection of organisms and epithelial abnormalities between two methods. Neutrophils, haemorrhage, mucus and debris were more in CPS than LBC (P value <0.05).

Conclusion: We didn't find significant difference between two methods in detecting cervical epithelial abnormalities. The high cost of LBC makes CPS still a better option in the countries with low resource setting.

Key-words: cervical cancer; conventional pap smear; human papillomavirus; liquid-based cytology

List of abbreviations

AGC Atypical Glandular Cells

ASCUS Atypical Squamous Cells of Undetermined Significance

ASCH Atypical Squamous Cells cannot exclude HSIL

BD Becton Dickinson

CIN Cervical Intraepithelial Lesion

CMC Chitwan Medical College

CPS Conventional Pap Smear

FDA Food and Drug Administration

HPV Human Papillomavirus

HPV DNA Human Papillomavirus Deoxyribo Nucleic Acid

HR-HPV High Risk Human Papillomavirus

HSIL High-grade Squamous Intraepithelial Lesion

IARC International Agency for Research on Cancer

LBC Liquid-Based Cytology

LSIL Low-grade Squamous Intraepithelial Lesion

MS-Windows Microsoft Windows

NAST Nepal Academy of Science and Technology

NILM Negative for Intraepithelial Lesion or Malignancy

Pap Test/Stain Papanicolau Test/Stain

P value Calculated Probability

SCC Squamous Cell Carcinoma

STI Sexually Transmitted Infection

SPSS Statistical Package for the Social Sciences

TBS The Bethesda System

AJCC American Joint Committee on Cancer

TNM Tumor Size, Regional Lymph nodes, Distant Metastasis

Introduction

Cervical cancer is the third most frequent cancer among women in the world, with an estimated 569,847 new cases and 311,365 deaths in 2018 [1]. The incidence of cervical cancer in Nepal is 19.3/100,000, making Nepal a country with one of the highest cervical cancer rates in South Asia. It is the first most common cancer among women in Nepal [2]. Papanicolau (Pap) test for cervical cancer screening has been well known technique. There are discrepancies of results between two cervical screening methods; liquid-based cytology (LBC) and conventional Pap smear (CPS). Sparse literatures from Nepal comparing these two screening techniques have been observed. The aim of our study was to compare the strength of LBC and CPS in detecting cervical dysplasia/cancer, and assess the feasibility of LBC in our setting. In view of importance of human papillomavirus (HPV) in the etiology of cervical cancer, we were also intended to perform HPV DNA (deoxyribonucleic acid) testing for detection of high risk-HPV (HR-HPV) in residual samples of LBC of patients, wherever possible.

Materials and Methods

Study Setting and Design

The present study was a cross-sectional study conducted at Department of Pathology, Chitwan Medical College Teaching Hospital, Chitwan from September 2018 to March 2019.

Ethical Approval

The study was approved by the Institutional Review Committee. An informed consent was taken from the patients.

Inclusion Criteria

All married women who visited for cervical screening were included. Samples from 312 patients were obtained.

Exclusion Criteria

Pregnant women, women who had history of hysterectomy, women who had received treatment for cervical intraepithelial neoplasia (CIN) and cervical cancer over the last five years were excluded from the study.

Data Collection Technique

Age, parity, literacy status, menstrual history, age of marriage, history of smoking, tobacco chewing, multiple sex partners, and oral contraceptive pills and their chief complaints were recorded in a Proforma.

Collection, Processing and Reporting of Samples

These samples were collected for both CPS and LBC for the comparative study from the same patients by scraping cells from squamocolumnar junction using Rovex Cervex Cyto brush. Split sampling technique was used in which material from one side of the brush was spread onto a clean slide and fixed by isopropyl alcohol fixative. Then the brush was dipped totally into a disposable LBC vial containing preservative fluid. After fixation, all the slides for CPS were stained by Pap stain. For LBC, the processing of samples and staining of smears was undertaken as per the prescribed protocol by BD SurePath™ method (LBC, BD Diagnostics, Becton, Dickinson and Company). CPS, LBC and biopsy reporting were done in our hospital, whereas residual samples of LBC were sent to a referral laboratory for HPV DNA testing to detect HR-HPV. Both CPS and LBC slides were analyzed by two consultant pathologists using an Olympus CX23 microscope and classified according to the Bethesda System (TBS) for Reporting Cervical Cytology 2014. The results of cervical Pap smears were correlated with follow-up cervical biopsies/resection specimens wherever available. Ancillary testing for HPV DNA for detection of at least one of 14 different types of HR-HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was performed in LBC residual samples of some patients those who had epithelial cell abnormality in Pap smears.

Statistical Analysis

Data analysis was done by using the statistical package for the social sciences (SPSS) version15 for Windows Microsoft Windows (MS-Windows) (SPSS Inc., Chicago, Illinois, the United States). Chi-square test and t-test were used for statistical evaluation and P value was calculated wherever required. P value ≤ 0.05 was considered as significance level.

Results

The present study included 312 women with the mean age 41.2 year (range 20-75 year). The major presenting symptom was whitish vaginal discharge, 101 (32.8%) followed by lower abdominal pain, 59 (19%) and itching vulva, 28 (9%). Other symptoms were burning micturition, per vaginal bleeding and postcoital bleeding.

Specimen Adequacy

CPS was satisfactory for evaluation in 296 cases while LBC was satisfactory in 304 cases with adequacy rate slightly higher in LBC (97.4%) than CPS (95%). However, there was no statistical difference in the adequacy rates between these two screening methods. Statistical calculation was done only on satisfactory smears for remaining parameters. Endocervical cells and metaplastic cells were also slightly higher in LBC than in CPS as shown in [Table 1].

Parameters	CPS n (%)	LBC n (%)	p-value
Adequacy			
-Satisfactory	296 (95)	304 (97.4)	> 0.05
Representative			
-Endocervical cells	219 (74)	232(76.3)	> 0.05
-Metaplastic cells	199 (67.2)	208(68.4)	
Background			
-Clean	33 (11.2)	68 (22.4)	< 0.05
-Inflammatory	249 (84.1)	233 (76.6)	
-Hemorrhage	14 (4.7)	3 (1)	
-Others (mucus, debris)	21 (7.1)	4 (1.3)	
Normal			
-NILM #	139 (47)	141 (46.4)	> 0.05
Reactive cellular changes			
-Inflammation associated	76 (25.7)	77 (25.3)	> 0.05
Epithelial abnormalities			
-LSIL #	1 (0.3)	1 (0.3)	> 0.05
-HSIL #	1 (0.3)	2 (0.7)	
-ASCH #	6 (2.0)	7 (2.3)	
-ASCUS #	19 (6.4)	19 (6.3)	
-AGC #	0	1 (0.3)	
Organisms			
-Candida	2 (0.7)	2 (0.7)	> 0.05
-Trichomonas vaginalis	0	2 (0.7)	
-Bacterial vaginosis	52 (17.6)	52 (17.1)	

Table 1: *Comparison between CPS and LBC in split samples*

AGC- Atypical glandular cells, ASCUS- Atypical squamous cells of undetermined significance, ASCH- Atypical squamous cells cannot exclude HSIL, CPS- Conventional Pap smear, HSIL- High-grade squamous intraepithelial lesion, LBC- Liquid-based cytology, LSIL- Low-grade squamous intraepithelial lesion, NILM- Negative for intraepithelial lesion or malignancy

Cellularity

Both methods revealed almost the same cellularity with superficial, intermediate and parabasal cells being the most common type of cells.

Background of Smears

CPS showed clean background in 33 (11.2%) cases only while LBC had clean background in 68 (22.4%) cases. Most of the CPS and LBC smears had inflammatory background [Table 1]. The cells were not well spread on CPS whereas a single layer of uniformly distributed cells was observed in LBC.

Diagnosis on Split Samples

The overall rates of negative for intraepithelial lesion or malignancy (NILM) were 139 (47%) in CPS and 141 (46.4%) in LBC. Two cases of Trichomonas infection and one case of atypical glandular cells (AGC) were diagnosed in LBC only. Epithelial cells abnormalities were detected in 30 (9.6%) cases either in both CPS and LBC or only in CPS or LBC samples. Out of 30 cases of epithelial cell abnormalities, 19 (63.3%) cases were reported as atypical squamous cells of undetermined significance (ASCUS); seven (23.3%) cases as atypical squamous cells cannot exclude HSIL (ASCH), one (3.3%) case as low grade squamous intraepithelial lesion (LSIL), two (6.7%) cases as high grade squamous intraepithelial lesion (HSIL); and one (3.3%) case as AGC. The pick-up rate for statistically significant epithelial cell abnormalities in split-samples was similar [Table 1].

Correlation of Pap Tests, Histopathology and HPV DNA Testing

Follow-up biopsies of four (1.3%) patients were received, thus histopathological correlation was possible in those cases only. Biopsy revealed three precancerous lesions (CIN I and CIN II), two of these cases were diagnosed as ASCUS and one as LSIL on Pap smears. Out of two HSIL, only one case had follow-up biopsy and histopathology revealed invasive squamous cell carcinoma (SCC). Later, total abdominal hysterectomy with bilateral salpingo-oophorectomy was performed in the same case [the AJCC stage (American Joint Committee on *Cancer)* was pT1NxMx (Pathologic, Tumor size, Regional lymph nodes, Distant metastasis)]. HPV DNA detection testing was performed in only ten cases whose Pap smear diagnosis was HSIL, LSIL or ASCH. HPV was detected in one case only with a diagnosis of LSIL on Pap smear. Biopsy revealed CIN I in that case. However, HPV was not detected in any cases of HSIL and ASCH [Table 2].

Epithelial cell abnormality	No. of cases (n=10)	Follow-up biopsy available	Histopathologic diagnosis	HPV DNA testing	
				Positive	Negative
ASCH # LSIL # HSIL #	7 1 2	2 1 1	CIN I& CIN II # CIN I # SCC #	0 1 0	7 0 2

Table 2: *Split samples reported as epithelial cell abnormality with follow-up biopsy and HPV DNA testing (n=10)*

ASCH- Atypical squamous cells cannot exclude HSIL, CIN- Cervical intraepithelial neoplasia, HPV DNA- Human papillomavirus deoxyribo nucleic acid, HSIL- High grade squamous intraepithelial lesion, SCC- Squamous cell carcinoma, LSIL- Low grade squamous intraepithelial lesion.

Discussion

Cervical cancer/dysplasia can be screened by CPS or LBC methods. The screening test should have both perfect sensitivity and specificity ideally. However, no such tests are known till date.

The sensitivity of CPS ranged from 57% to 74% and 61% to 73% for LBC, while the specificity ranged from 91% to 96% for CPS and 90% to 95% for LBC in the study conducted by Coste et al [3]. A meta-analysis by Abulafia et al. did not show significant differences in sensitivity and specificity between CPS and LBC in most of the studies [4]. The similar finding was observed by Arbyn et al [5]. However, Hussein et al. observed very high sensitivity and lower specificity of LBC [6]. The high specificity of LBC was discussed elsewhere [7,8]. Chinaka et al. found 100% sensitivity and specificity of LBC [9]. The sensitivity and specificity of LBC vs CPS were 100% and 81.8% vs 88% and 99% in the study conducted in Nepal [10]. This shows high discrepancies of sensitivity and specificity between these two cervical screening methods and it is very difficult to choose one as an important tool for screening of cervical cancer. We could not analyze the specificity and sensitivity of these two methods because biopsies of all the subjects with epithelial cell abnormalities on Pap smears were not available for histopathological evaluation which is used as the gold standard technique. The present study had 30 (9.6%) cases of epithelial cell abnormalities. However, follow-up biopsies of only four (1.3%) subjects were available. Histopathology revealed one each case of CIN I and CIN II, diagnosed both as ASCH on Pap smear and one case of CIN I, diagnosed as LSIL on Pap smear. Only one out of two cases reported as HSIL on Pap smear had follow-up biopsy that revealed SCC.

Several studies reported that LBC had more satisfactory smears when compared to CPS [9, 11-15]. The statistically significant difference between rates of adequacy between two methods was observed elsewhere [8, 14, 16]. In contrast the reports by Davey et al., Sharma et al., and our study showed no significant difference in the rates of adequacy between CPS and LBC [17,18]. Specimen adequacy is highly improved on LBC due to absence of obscuring factors in the background like inflammatory cells, blood and mucus or inappropriate spreading and fixation of cells. Drying artifacts and cytolysis is minimal or absent and there is uniform distribution of cells on LBC. This study also noted cleaner background with lesser number of cases showing inflammation, hemorrhage, mucus and debris on LBC ($P < 0.05$).

The present study implemented spilt sampling technique like other studies [13, 14]. In contrary, others had separately collected samples involving the direct vial transfer for LBC and Ayre spatula for CPS [10, 18]. Although some authors had adopted spilt sampling method unsatisfactory rate was far less in LBC samples in their research, [13, 14] however; specimen adequacy was not improved in LBC in the present study. We assume that due to split sampling method, there was an initial distribution of cells in conventional smears and ultimately transfer of samples in the LBC vials might have caused limited epithelial cells on LBC. This must have lead to almost equal number of unsatisfactory smears in both techniques in our study.

This study showed no significant difference in the presence of transformation zone component of endocervical cells/metaplastic squamous cells in CPS and LBC. However, Strander et al. mentioned that most LBC smears had no endocervical cells when compared to CPS.[19] In contrary, other authors found more number of endocervical cells in LBC [18,20]. Sharma et al. had explained the reason behind their findings was because of the cleaner background of LBC smears allowing a better visualization of the transformation zone component and the fact that there is a chance of loss of some cells in the collecting device of CPS whereas direct transfer of the entire collecting device for the preservation in LBC method, it is likely to be more representative and it allows homogenization of the sample during processing as well [18].

There was no significant difference in detection of reactive changes between the two methods in our study. Similar finding was noted by other study [18]. The detection of pathogenic organisms was more in LBC in the study of Sherwani et al [7]. In contrary, Sharma et al. found that organisms were better picked up in CPS [18]. This study did not show significant difference in the picking up pathogenic organisms between CPS and LBC.

Several studies showed no significant difference between two methods in detecting cells ranging from normal to HSIL which is compatible to our study [12, 20-22]. Some authors detected ASCUS more frequently with CPS although there was no significant difference in detection of LSIL/HSIL [17, 21]. However, increased detection rate of ASCUS with LBC was discussed elsewhere [11, 20]. Many scholars analyzed that detection of LSIL and HSIL was more in LBC when compared to CPS [4, 7, 23]. The detection rate of LSIL and HSIL was increased by 47% in SurePath Pap ($P = .0011$) and 116% ($P = .0002$), respectively when compared to CPS [24].

The present study included comparison between SurePath and conventional Pap methods similar to the study by Sharma et al [18]. Some authors compared ThinPrep Pap to conventional Pap test.[4,23] Many included multiple technologies in their studies (such as ThinPrep Pap test, SurePath Pap test, and/or other technologies not approved by the FDA) [5, 17].

Pradhan et al. and Kang et al. had compared HPV DNA testing and Pap smear as cervical screening tools [25, 26]. According to Pradhan et al. HPV testing had greater sensitivity for detection of CIN II and CIN III than Pap test [25]. In contrary, an 11-year retrospective study conducted in Korea by Kang et al. demonstrated that Pap smear had greater sensitivity and specificity for detecting HSIL and SCC when compared to HPV testing [26]. Pap smear also appeared more promising than HPV testing to detect glandular lesions. HPV testing and Pap smear did not differ significantly for detecting LSIL in their study. Though HPV testing

alone proved to be promising for primary cervical screening in the study conducted in India by Pradhan et al., the study was conducted in a small sample size [25]. The cost effectiveness is the limitation for implementation of HPV testing in cervical cancer screening in the countries like India and Nepal with low resource setting. LBC with concomitant HPV testing may be applicable and more effective in high-resource setting.

A study conducted by Johnson et al. demonstrated 9.6% HR-HPV, which was the first study to assess HR-HPV among rural Nepali women from Far Western Nepal using clinician- and self-collected cervical sampling methods [27]. Among 8 women with abnormal cytology, one woman had SCC and seven women had HSIL. HR-HPV was detected in seven (87.5%) in clinician collected samples and six (75.0%) in self-collected samples among these eight women. Pankaj et al. performed HPV DNA testing in all patients along with comparison of CPS and LBC [14]. Their study detected HPV DNA for high-risk oncogenic types in 6.45% of women studied and 5.37% of women with normal cytology. The prevalence of HPV 16/18 among women with normal cytology is 3.9% worldwide [1]. This indicates that HPV DNA testing must be performed in samples of all subjects as HR-HPV was detected in normal cytology as well, if possible.

Bhusal et al. studied of high-risk HPV oncogenic types in a smaller number of Nepali women diagnosed with invasive cervical cancer [28]. HPV16 was the most common HR-HPV (50% of HR-HPV) followed by HPV18 (18% of HR-HPV). A study performed by an International Agency for Research on Cancer (IARC) reported 8.6%, 6.1%, and 1.9% of any HPV, HR-HPV, and HPV16, respectively among women from a general population in the South central part of Nepal, Bharatpur [29]. The present study performed HPV DNA testing in only 10 patients with diagnosis of epithelial cell abnormalities on Pap smears. The positive result was detected in only one patient with LSIL in Pap smear. Seven cases of ASCH and two cases of HSIL (one of which revealed SCC in biopsy) in Pap smear were negative for HPV. The cause of HPV negative in these cases might be due to a low viral load or HPV-negative SCC [30].

There are some limitations in the present study. This study was a hospital based study, hence there was a limited number of cases with relatively small sample size and did not represent a particular community. Broad spectrum multi-centric and community based studies should be conducted to estimate the actual burden of cervical cancer in Nepal. We observed almost equal number of unsatisfactory smears on CPS and LBC smears. The reason behind this may be due to split sampling technique. HPV DNA testing could not be performed in all cases though ideally, it should be performed in all residual LBC samples to identify the exact incidence of HR-HPV infection of cervix. Follow up biopsies of all patients with epithelial cell abnormalities were not available for further histopathological evaluation which is used as the gold standard technique, thus specificity and sensitivity of two methods could not be analyzed.

Major advantages of LBC were cleaner background without mucus and debris, less number of neutrophils and red blood cells. However, there was no significant difference between CPS and LBC in the detection of epithelial cell abnormalities, and also the high cost of LBC makes CPS still a better option in the countries with low resource setting like Nepal.

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Conflict of Interest

We declare that there is no conflict of interest.

Author's Contributions

Sushna Maharjan: conceptualization, funding acquisition, visualization, data analysis, draft preparation and review.

Mamata Tiwari: formal analysis, critical review and editing.

Both authors have read and approved the final version of the manuscript.

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