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The Utility of a Manual Liquid Based Cytology in Screening for Pre-Cancerous Lesion and Cervical Cancer

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Abstract
Introduction: *Liquid-based cytology is a technique that enables cells to be suspended in a liquid medium and spread in a monolayer, thereby enabling a better morphological assessment. Automated techniques have been widely used especially in the developed countries but limited in the developing countries due to cost and availability.*
Conventional Pap smear (CPS) examination has been the commonly used method for detection of cervical cancer. However, there have been challenges in its use due to the inherent limitations, like presence of obscuring blood and inflammation which has reduced its sensitivity considerably. On the other hand, manual liquid based cytology (MLBC) is a technique that is cost effective and improves detection of precursor lesions and specimen adequacy.
Methodology: *A total of 295 women were assessed for pre-cancerous lesions and cervical cancer using Manual Liquid Based Cytology and Conventional Pap Smear method.Cohen Kappa test was run to determine the level of agreement the two methods.*
Results: *There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065). Specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. There was increased detection rate of abnormal cervical cytology smears with MLBC of 85.7%.*
Conclusion: *Manual liquid based cytology was found to give better results than conventional Pap smear and therefore it can be used as an alternative liquid based cytology technique for cervical cancer screening in limited resource settings.*
Keywords: *Manual Liquid Based Cytology, Conventional Pap Smear.*

Introduction

The introduction of cytologic screening for cervical cancer using the Papanicolaou (Pap) test in the 1950 has led to a reduction in the incidence of invasive cervical cancer in the developed countries. This has been attributed to the effective

screening and treatment programs in these countries[1]. However, the establishment and implementation of Pap smear programs have not yet been possible in the developing countries and thus cancer of the cervix continues to threaten the lives of women from these countries up to date. Developing countries have lagged behind in combating the high mortality rate of cervical cancer because of limited resources and lack of a well-funded healthcare system[2,3].

Conventional Pap smear (CPS) examination has been the commonly used method for detection of cervical cancer. However, there have been challenges in its use due to the inherent limitations which include: Majority of cells not captured as only a portion of the sample is smeared onto a microscope slide after collection. Furthermore, there is no representative transfer of the sample as the collection device is discarded, sometimes with more than 80% of the patient's sample still on the device. [4,5]

Another problem associated with CPS is clumping and overlapping with more than one layer of cells formed leading to a poor visualization of the cells. The conventional Pap smear specimen may often be clouded with debris such as blood and mucus, which obscures cell visibility[5] Drying artifacts, may also be formed if the cells are not fixed immediately. Lastly, the collection device is discarded and thus a repeat sample is not available incase needed. These limitations have been shown to reduce the sensitivity of CPS to less than 50% [5, 6]

An automated liquid-based cytology (ALBC) for cervical cancer screening was developed to improve the sensitivity, but the very high cost related to automated devices has hampered its implementation in the setup of developing countries, like Kenya.

A screening technology that matches the limited resources we have in Kenya will be of benefit both to physician and the patient.It is in this regard that the current study was done to develop and evaluate the utility of a low cost manual liquid based cytology method in screening of pre-cancerous and cervical cancer. This was expected to increase the survival of cervical cancer patients and thus improving their health condition and quality of life.

Methodology

The present study was carried out at Machakos Level 5 Hospital of Machakos County in South - Eastern part of Kenya. The study was done at the comprehensive care Centre which serves around 2000 women infected with HIV aids. Ethical approval was obtained from the Kenyatta University Research and Ethical Review committee (Protocol Number: PKU/464/E42). The population of the present study was composed of women of 18 years and above who were sexually active and attending Machakos level 5 Comprehensive Care Centre (CCC).Two hundred and ninety five women were included in this study.

Women who were pregnant or declined to complete an informed consent form were excluded from the study. Women on treatment for precancerous lesion or cervical cancer and a previous hysterectomy were not eligible. Convenience sampling method was used, with this method, patients available during the study period and willing to participate were considered until the sample size was reached. Sampling was done until the intended sample size was obtained. After obtaining a signed consent from the patient, the clinician explained the procedure, assured and placed the patient in a comfortable and convenient position for sample collection. First sample was collected using cytobrush for conventional Pap smear. The cytological material obtained was spread on glass slides directly and fixed immed-iately in 95% ethanol for at least 15 minutes.

A second sample was collected using a different cytobrush. The cytological material was transferred with brushes into a formulated liquid fixative (containing sodium chloride, sodium citrate, 10% formalin and isopropyl alcohol). Brushes were broken off into the container of collection fluid. The collected samples were

vortex-mixed, and then about 10ml transferred into a formulated alcoholic-agar (polymer solution containing agarose, polyethylene glycol, poly-l-lysine and alcohol) in nipple-bottom test tubes. Test tubes were centrifuged for 10 minutes at 2000rpm. The supernatant was discarded and from the deposit smear made on a clean glass slide using a Pasteur pipette. The prepared slides were fixed by drying them in a hot air oven for 15 minutes at 50˚C. The slides were further fixed by dipping them in 95% alcohol for 15 minutes. All the smears were stained using the Papanicolaou staining method.

All the Pap smears were screened by the principal investigator and signed out together with a pathologist. The Bethesda system 2014 for reporting cervical cytology was used for reporting all the cytological abnormalities observed during examination and reporting. All abnormal cytological smears of the study patients were blindly and independently re-evaluated by a board certified pathologist. The abnormal smears were triaged for further investigation**.**

Cohen's kappa test was done to determine the agreement between CPS and MLBC. Increased detection rate (IDR) was calculated as follows: IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

Results

Table 1 Pap smear findings as seen in the conventional Pap smear method

Pap smear Findings	Frequency	Percent
NILM	259	87.8
Abnormal smear	14	4.7
Unsatisfactory smear	22	7.5
Total	295	100.0

Table 1 shows the Pap smear findings as seen in the conventional Pap smear method. Out of the total women recruited in this study, 259 (87.8%) were Negative for intraepithelial lesion or malignancy, 14 (4.7%) had abnormal smears while 22 (7.5%) had unsatisfactory smears for evaluation. A total of 295 women were enrolled in the study. Twenty two were excluded from the analysis due to inadequate smear or missing cervical cells at all. The prevalence of cervical cytology abnormalities as seen by the conventional Pap smear method in this research was 14 out of 273 (5.1%) with HSIL being the most prevalent at 5 out of 273 (1.8%), ASC-H 4 out of 273 (1.46%), LSIL 3 out of 273 (1.05%), SCC was seen in 1 out of 273 (0.36%) and lastly Adenocarcinoma was also seen in 1 out of 273 (0.36%).

Table 2 Pap smear findings as seen in the MLBC method

Pap smear Findings	Frequency	Percent
NILM	257	87.1
Abnormal smear	26	8.8
Unsatisfactory smear	12	4.1
Total	295	100.0

Table 2 shows Pap Smear Findings using the Manual Liquid based cytology Method. Out of the 295 Pap smear samples screened using the manual liquid based cytology technique, 257 (87.1%) were Negative for intraepithelial lesion or malignancy, 26 (8.8%) had abnormal smears while 12 (4.1%) had unsatisfactory smears for evaluation. LSIL was the most prevalent at 9 (2.7%), HSIL 7 (2%), ASC-H 5 (1.6%), ASCUS 3 (1%), Adenocarcinoma 1 (0.3%) and squamous cell carcinoma 1(0.3%)

Table 3 Cross tabulation of Conventional Pap smear and Manual Liquid based cytology results

		MLBC			Total	kappa	P value
		NILM	Abnormal	Unsatisfactory			
CP	NILM	247	12	0	259	0.673	0.065
	Abnormal	0	14	0	14		
	Unsatisfactory	10	0	12	22		
Total		257	26	12	295		

Table 3 shows Cross tabulation of Conventional Pap smear and Manual Liquid based cytology results. There was moderate agreement between the two methods (k=0.673, 95% CI, p=0.065).

Comparison of cytomorphological patterns between Conventional Pap smear and Manual Liquid based cytology

Figure 1 High-Grade Squamous Intraepithelial Lesion on MLBC

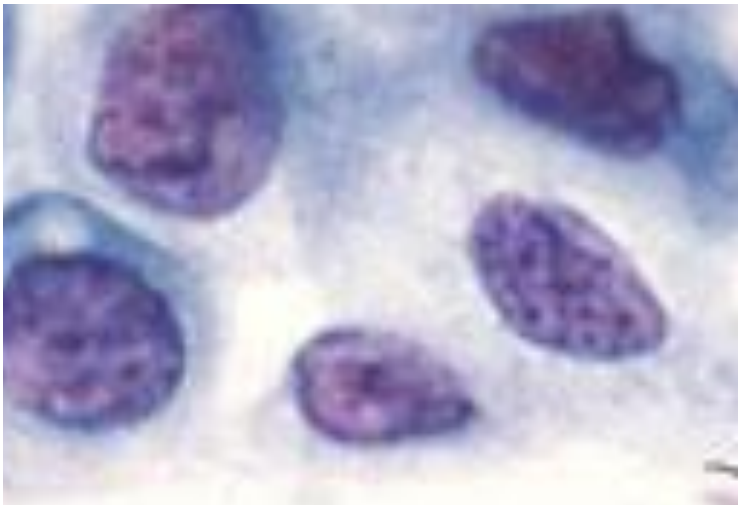
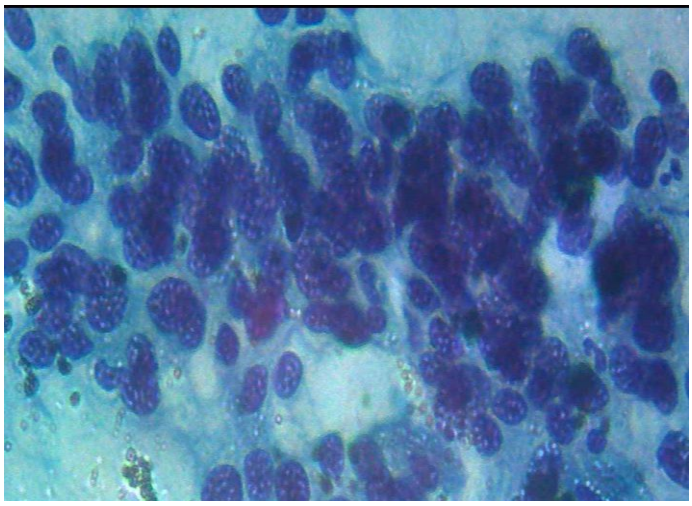


Figure 2 High-Grade Squamous Intraepithelial Lesion on MLBC



With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy (Figures 1 and 2**)**

Increased detection Rate

Increased Detection Rate with (IDR) was calculated as follows;

IDR= ((Pm-Pc)/Pc)*100, where Pm is the number of positive cases through MLBC and Pc is number of positive cases for conventional Pap smear.

$$IDR = \frac{(26-14)100}{14}$$

$$= 85.7\%$$

Thus Increased detection rate with MLBC in this study was 85.7%

Discussions

In this study, specimen adequacy was found to be better with MLBC than CPS with 12 unsatisfactory smears in MLBC and 22 in CPS. Many studies have reported LBC to be better in terms of specimen adequacy compared to CPS. Majority of studies comparing LBC and CPS found that the quality of slides improved in LBC, which is consistent with the results obtained in our study that MLBC has higher satisfactory specimen rates as compared to CPS.

With the MLBC technique, majority of the smears showed cells suspended in a monolayer thus improved detection of precursor lesions and improvement of specimen adequacy. A clear background was also observed for the MLBC smears with minimal obstruction of cells by debris, mucus or blood further improving the specimen adequacy. In a study done by Kavatkar et al. (2008), MLBC was found to give a clear background in more smears than in conventional Pap smear.[6]

In CPS, there was higher rate of unsatisfactory smears due to the presence of obscuring blood, inflammation and dirty background which obscured the epithelial cells thus affecting the screening process. Also, in CPS only 20% of the cells collected on the brush are smeared on to the slide leading to lesser cells being transferred to the smear for screening thus unsatisfactory smears for evaluation[7]. Specimen adequacy in MLBC was also better compared to CPS due to the fact that the entire specimen collected from the cervix was transferred to the fixative solution for processing without any wastage.

In the current study 7.5% cases were unsatisfactory with conventional Pap smear method while with MLBC, 4.1% cases were found unsatisfactory due to inadequate cells for examination. A study done in Pakistan to compare cervical cell morphology using MLBC and CPS recorded 27% cases of unsatisfactory smears for conventional Pap smear method and 24% with the MLBC technique[9]. Other studies have documented lower percentages for unsatisfactory smears with LBC technique. Bergeron et alreported (0.14%) while Garbar et alfound (0.9%). In these studies, automated liquid based cytology technique was used thus the lower percentages of unsatisfactory smears compared to the current study. [12,13]

In LBC, the sample is first placed in fixative solution followed by further processing instead of making slides directly as in CPS. This makes cellular structure better preserved with reduced drying artifacts as cells are immediately fixed [11, 14]. In MLBC there is marked decrease in artifacts, contaminating mucus and blood. Cells are evenly distributed on slides and centrifugation in this method offers a proper mixing[15]. Similarly, in the current study cellular overlapping is reduced with majority of the cells forming a monolayer with a clear background. Thus manual liquid based cytology gives more clear results with clear background, less artifacts and lesser degree of cellular overlapping when compared with conventional Pap smear.

In the current study, the level of agreement between MLBC and the conventional Pap smear method was 67.3%. This is comparable to 68% as documented in a study done in India by Nandini et al[8]. In another study done in India on manual method of liquid-based cervical cytology by Kavatkar et al, the level of agreement between MLBC and the conventional Pap smear method was found to be 88%[6]. However, in another study done by Moosa et al, conventional Pap smear method was found to be comparable to the MLBC technique in terms of specimen adequacy and detection of abnormal cervical cytology[9]. In the current study, there was increased detection rate of abnormal cervical cytology smears with MLBC of 85.7%. However, this is low compared to 150% as documented in a study done by Nandini et al[8]

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