

Liquid Based Cytology versus Conventional Pap for Evaluation of Cervical Smear among Reproductive Women, Wad Medani, Gezira State, Sudan, 2023

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Abstract: Introduction: Cervical cancer is the second most common cancer among Sudanese women preceded by breast cancer. Cervical cytology “by its two branches conventional pap (CP) and Liquid Based Cytology (LBC)” is a stepping stone in cervical cancer prevention and cure. **Aim:** This study aimed to compare between two collection and preparation methods of cervical cytology, conventional Pap smear (CP) and manual LiquiPREP™ (LP), for evaluation of cervical smear among Sudanese reproductive Sudanese women. **Methodology:** The study included a total of 137 Sudanese reproductive women attended to Wad Medani Obstetrics and Gynecology Teaching Hospital and some private clinics during the period from February 2020 to January 2023. Both manual LP Smears and CP smears were prepared and evaluated according to The 2014 Bethesda System for Reporting Cervical Cytology. **Result:** From 137 CP and 137 LP, satisfactory smears were 134 and 136 respectively. LP improved background clarity ($P = 0.001$) and provided evenly distributed cellular pattern ($P = 0.000$). Pathogenic microorganisms were identified in 23 CP and in 22 LP smears. Cytomorphological findings were relatively similar in both CP and LP ($P = 0.536$). **Conclusion:** LP reduced U/S (unsatisfactory/satisfactory) rate, improved background clarity and provided evenly distributed cellular pattern over CP. Detection of microorganisms and cytological changes was relatively similar in both CP and LP.

Keywords: conventional pap, Liquid-based cytology, LiquiPREP™, cervical cancer, Sudan

Introduction:

Worldwide, cervical cancer is considered as a second most important women cancer preceded by breast cancer. According to GLOBOCAN estimates for cancer in Sudan (2020), cervical cancer incidence among all age Sudanese females was 1227 (4.5%), cancer death was 828 (4.9%) and five years prevalence was 2227 (1). Since more than two-thirds of all Sudanese women with invasive cervical cancer are diagnosed at an advanced stage, early detection of precancerous lesion can help in disease prevention and this can be achieved through screening programmes (2).

Cervical cytology is the most widely used screening method that has been use for more than fifty years and based on detection of shaded abnormal epithelial cells. Two techniques for cervical cytology has been used currently, conventional pap smear and the newer Liquid Based Cytology (3).

Regular Pap test is very important to perform because most invasive cervical cancers are found in women who have not had regular Pap tests (American Cancer Society, 2020). Conventional pap test is the most frequent and most cost-effective method for cervical cancer screening, diagnosis, prognosis and follow-up and it decreases the morbidity and mortality rates for cervical cancer specially when co-testing with human papillomavirus (HPV) DNA testing (4). Pap smear is used specifically throughout cervical cancer screening process to collect exfoliated cells to be checked at closely within the lab for the detection of both precancerous and cancerous changes in cervical canal of the uterus rather than endometrium and vagina (5).

Liquid Based Cytology (LBC) is a recent and increasingly being used technology that represents a major change in collection, preservation and preparation of samples for cervical screening. The liquid-based cytology was firstly approved by Food and Drug Administration (FDA) in 1996. The technique showed up many advantages over conventional pap; it reduces debris and cell overlapping and it makes the specimen homogeneous and monolayer, and in addition, it increases specimen's adequacy resulting in uniform cell distribution in a smaller screening area and shows cleaner background which in turn reduces slide screening time (6). LBC removes air-drying artifact due to immediate liquid fixation and it is also improves diagnostic accuracy through enhancing cellular and nuclear details. LBC residual material can be successfully used for advance ancillary studies such as cell blocks “which provides additional diagnostic information including architectural pattern”, immunocytochemistry, in situ hybridization, special stains, DNA and other molecular tests. Furthermore, liquid-based preparation (LBP) can be used for any ancillary studies by preparing more additional slides from the specimen vial (7). Manual Liqui-PREP™ protocol is very simple and does not require special imaging equipment like other liquid based technologies “ThinPrep™ and Surepath™” which require an expensive processor systems and occupy a large area in the laboratory. LiquiPREP™ is also relatively cost effective technique when comparing with more expensive automated techniques (8).

Materials and Method:

The current study is a cross sectional study performed among 137 reproductive women “15-49 years” attended to *Wad Medani* Obstetrics and Gynecology Teaching Hospital and some private clinics for gynecological consultation during the period from February 2020 to January 2023. The study included married, non-pregnant and non-menstruating reproductive women signed on an informed consent for participation in the study after reviewing a summary from the study plan. Ethical approval was obtained from Ministry of Health in Gezira state, Sudan.

Pap smear collection and preparation: The samples were collected by a consultant gynecologists using a medium size, sterile, disposable plastic speculum and cervical brush/broom. CP was firstly smeared on a labelled frosted glass slide and immediately fixed in 95% ethanol for 15 minutes. Then the head of the brush was detached and immersed in a labelled Liqui-PREP™ preservative vial and allowed at least for 1 hour before processing in the laboratory. According to manufacturer’s protocol for Liqui-PREP™ sample preparation, samples were mixed and centrifuged in 10.000 rpm for 10 minutes. After discharging the supernatant, the pellet was suspend in cellular base in a dilution of 1:3 and then the mixture was vortex. About 50 µl from the mixture was smeared manually on labelled frosted glass slide and allowed to dry for 1 hour. A total of 274 cervical smears (137 CP and 137 LP) were stained by Papanicolaou staining method and evaluated according to The 2014 Bethesda System for Reporting Cervical Cytology.

Data collection and analysis: Data were collected by well-constructed questionnaire included all required information and statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software programme version 20 and Microsoft Excel.

Results:

The study included a total of 137 reproductive women of varying ages between 15 to 49 years old attending *Wadmedani* Obstetrics and Gynecology Teaching Hospital and some private clinics. Half of the participants (46%) were between 26 - 36 years old and the majority (57.7%) were rural residents.

69.3% of participants complained of vaginal discharge with odor or odorless while 4.4% complained of abnormal bleeding and 26.3% were apparently healthy. A total of 274 cytology smears (137 CP and 137 LP) were evaluated firstly for specimen adequacy and quality of smear. 97.8% of CP and 99.3% of LP smears were satisfactory for evaluation. LP smears showed more clear background than CP smears ($P = 0.001$). Distribution of cell in LP smears was more evenly than in CP ($P = 0.000$) [Table 1].

Pathogenic microorganisms (*Gardnerella Vaginalis*, *Candida spp*, *Trichomonas Vaginalis* and Koilocytic changes of *Human Papilloma Virus*) were identified in 23 CP and in 22 LP smears [Table 2].

The cytological results were classified as Negative for intraepithelial lesions or malignancy (NILM), atrophy, atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H), Atypical glandular cells (AGC) and Endocervical Adenocarcinoma [Table 3].

Table 1: Comparison of specimen adequacy and smear quality between CP and LP smears:

Items		Technique		p.value
		CP	LP	
Specimen Adequacy	Satisfactory	134 (97.8%)	136 (99.3%)	0.311
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
Background	Clear	62(45.3%)	95 (96.3%)	0.001
	Inflammatory	54 (39.4%)	33 (24.1%)	
	Hemorrhagic	18 (13.1%)	8 (5.8%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
T.Z	Endocervical	46 (33.5%)	47 (34.3%)	0.625
	Squamous metaplastic cells	26 (19.0%)	26 (19.0%)	
	Both	20 (14.6%)	14 (10.2%)	
	Absent	42 (30.7%)	49 (35.8%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
Cell distribution	Even	73 (53.3%)	124 (90.5%)	0.000
	Overlapped	61 (44.5%)	12 (8.8%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
Nuclear details	Good	118 (86.1%)	120 (87.6%)	0.601
	Poor	16 (11.7%)	16 (11.7%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
Stain quality	Good	108 (78.8%)	115 (83.9%)	0.417
	Poor	26 (19.0%)	21 (15.3%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	

Cell appearance	Good	112 (81.8%)	118 (86.1%)	0.459
	Poor	22 (16.1%)	18 (13.1%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
	Total	137 (100%)	137 (100%)	

Table 2: Distribution of pathogenic microorganisms in CP and LP smears:

Items		Technique		P value
		CP	LP	
Microorganisms	<i>Gardnerella Vaginalis</i>	11 (8.0%)	11 (8.0%)	0.532
	<i>Candida spp</i>	9 (6.6%)	10 (7.3%)	
	<i>Trichomonas Vaginalis</i>	2 (1.5%)	0	
	<i>Human Papilloma Virus</i>	1 (0.7%)	0	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
	No microorganisms	111 (81.0%)	115 (84.0%)	
Total		137 (100%)	137 (100%)	

Table 3: Cytomorphological findings in CP and LP smears:

Items		Technique		P value
		CP	LP	
Cytomorphological findings	NILM	126 (92%)	130 (94.9%)	0.536
	Atrophy	3 (2.2%)	3 (2.2%)	
	ASC-US	3 (2.2%)	0	
	ASC-H	0	1 (0.7%)	
	AGC	1 (0.7%)	1 (0.7%)	
	Adenocarcinoma	1 (0.7%)	1 (0.7%)	
	Unsatisfactory	3 (2.2%)	1 (0.7%)	
	Total	137 (100%)	137 (100%)	

Discussion:

For the last five decades, Pap test was an accredited method for cervical cancer screening. Cytological smears were applied as an ideal screening test for precancerous lesions which if treated, would not be developing into cervical cancer (9).

LBC is a newly advanced collection and preparation technique taking place in routine practice in many countries. It's a technique that preserved the cells in a monolayer with relatively clear background for a long time to be accessible when needed (10).

The current study was a cross sectional study aimed to compare between CP and LP smears to evaluate the utility of LP over the CP in cervical cytology. We aimed to compare the specimen's adequacy, quality of smear and detection rate for pathogenic microorganisms rather than cellular changes between CP and LP to justify which is better for screening, diagnosis and for routine practical.

Among 137 CP and 137 LP evaluated smears, satisfactory CP smears were 97.8% while satisfactory LP smears were 99.3% similar to a study conducted by Aksamentov *et al.* (11) and noted that LBC over CP improves the specimen adequacy and reduces U/S rate because of increasing the quality of smears for diagnosis.

Transformation zone cells were detected in CP (67.1%) more than LP (63.5%), that was in agreement with a study done by Campaner and Fernandes (12) and found that conventional cytology was associated with a higher representativeness of the TZ cells than LBC. Furthermore, LP was significantly improved background clarity ($P = 0.001$), and provided an evenly distributed monolayer cellular pattern ($P = 0.000$) because it removed obscuring hemorrhagic and inflammatory backgrounds and reduced cellular overlapping similar to a study conducted by Gupta *et al.* (13) and to a study conducted by Khakwani *et al.* (14) and both noted that LBC is better in terms of adequacy of smear in compare with CPS.

Identification of pathogenic microorganisms was statistically the same by both techniques CP and LP ($P = 0.532$) with relatively higher detection for *Candida spp* by LP and higher detection for TV and HPV by LP. In our study we found that Bacterial Vaginosis is the most common among cervico-vaginal inflammatory disease followed by *Candida* infection, Trichomoniasis and HPV infection. GV, *Candida spp* was more detected in LP but TV was easier detected in CP. That was in disagreement with a study done

by Kaza *et al.* (15) and found that Bacterial vaginosis followed by *Candida* hyphae, TV, *leptothrix* and *Herpes Simplex Virus* (HSV) inclusions were more easily detected on LBC smears compared to CP smears.

In this study, we showed that cytomorphological findings in both evaluated CP and LP smears were statistically similar ($P = 0.536$). But we found that ASCUS smears were detected in CP only 3(2.2%), while interpreted as NILM in LP and that's mean that LP decreased the ASCUS ratio. Our findings were in agreement with a study done by Erdogan *et al.* (16) and noted that LBC decreases the ASCUS ratio than CPS.

ASC-H was detected in one LP smear. That was in agreement with a study done by Modi and Modi (17) and found that LBC is better than CP test in detecting squamous precancerous lesions. Also endocervical adenocarcinoma and AGC were detected similarly in both CP and LP smears and that was in agreement with a study conducted by Neville and Quinn (18) and found LBC and CP had similar detection rates of HSIL, cervical squamous cell carcinomas, and adenocarcinomas.

Conclusion:

In conclusion LP seems to be an ideal technology to improve specimen adequacy and to provide good quality of smear than CP. Despite LP reduces the ASCUS rate comparing with CP, both technologies had statistically similar cytomorphological finding.

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Conflict of interest:

Authors have nothing to declare.

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