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Khalid Ahmed Khalid Alama, MSc.
Prof/ Adam Dawoud Abakar

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Khalid Ahmed Khalid Alama, MSc.
khalidalama@gmail.com
Dubai Hospital- UAE.

Prof/ Adam Dawoud Abakar
Head of Medical parasitology department- University of Gezira

Abstract

The main aim of the present study was to assess the diagnostic accuracy of Liquid base versus Conventional smears (CS). The specific objective was to evaluate and compare efficacy liquid base cytology with conventional cytology (CS) as a screening tool and to assess the quality of immunohistochemical stain in conventional smears. A prospective study including 100 cervical samples over a period of six month. Split sample was obtained using cervex-brush. CS was prepared from the brush and the brush head was suspended in the LBC vial and processed by thin prep 5000 machine. The smears were stained with Pap stain and extra five conventional and thin prep slides prepared and stained with immunomarker. Results showed that there were 4.0% unsatisfactory (U/S) cases in CS and 1.0% in LBC; the main cause was ranging between obscuring blood and inflammation in CS and low squamous cellularity in LBC. About 5% split samples had epithelial abnormalities both in CS and LBC (3% atypical squamous cells of undetermined significance (ASCUS), devided between LBS 2% while CS1%.Low grade squamous intraepithelial lesion (LSIL) 2%, devided between LBC 1% and CS 1%. Infections as Trichomonas vaginalis (TV) and spores of candida species, 1% and 2% respectively detected only in LBC smear and missed in CS preparations of the same samples, considering 2-3 minutes for LBC screening and 5-6 minutes for CS screening following the international standards. Conventional smears did not appear to confer a cytomorphological advantage and has a lower diagnostic accuracy using IHC. The sensitivity of Thin Prep was significantly higher than that of CS due to cellular clumps and presence of marked inflammatory cells and blood which compete other epithelial cellular elements in staining affinity in addition to the length of the smear which need large volume of stains to cover the whole area. While the confined area of thin prep smear and homogenous cellular distribution support the advantages of thin prep over the conventional smear when using IHC stain. The study concluded that LBC technique leads to significant reduction of U/S rate. LBC samples offered better clarity, uniform spread of smears, less time for screening and better handling of hemorrhagic and inflammatory samples. In addition to feasibility to do further special stains and HPV tests. LBC had equivalent sensitivity and specificity to CS.

Keywords: *Cervical cancer, cervical precancer, liquid base cytology and conventional smear*

INTRODUCTION

Liquid-based cytology (LBC) was introduced in mid-1990s as an alternative technique to process cervical samples. Since then a lot of countries in the Western world has switched from conventional Pap smear (CS) method to LBC, although amid contrasting results from various studies comparing the benefits of LBC with CPS. LBC is proposed to have many benefits over CS such as less number of unsatisfactory (U/S) smears [1],[2]. More representative transfer of cells from collecting device evenly distributed cellular material, the choice of using residual cellular material for human papillomavirus (HPV) testing, reduced screening time and possibly higher rate of high grade squamous intraepithelial lesion (HSIL) detection. Extra slides prepared from residual LBC material has been shown to upgrade the diagnosis in 14.3% cases. [3]. Most consistent benefit of LBC over CS observed in various studies is reduced rate of U/S smears. [10],[11],[12],[13] . Diagnostic accuracy of LBC when compared to CS is a matter of great debate. Several studies have shown increased sensitivity of LBC over CPS, [14],[15],[16[,[17] whereas others showing decreased or equal sensitivity and specificity. [18[,[19],[20] Previous studies have also shown increased detection of glandular abnormalities in LBC preparations. [21]. The present study was undertaken to study the differences between conventional and LBC methods in cervical Pap samples and to assess diagnostic accuracy.

MATERIAL AND METHODS

The study was a prospective study comprising of 100 consecutive cervical samples from women visiting the of Obstetrics and Gynecology Department over a period of six month. The samples taken were part of routine hospital-based screening of patients for cervical epithelial lesions. The speculum used to visualize the cervix and samples were taken with cervex-brush and divided into two parts (split-sample technique). First, a CS was prepared and was immediately fixed on 95% ethyl alcohol. After that same brush head was suspended in LBC vial containing preservative fluid, which was transferred to the cytopathology laboratory for further processing using thin prep 5000 processor machine. Pap stain used to stain the smears as follows:

1. 95% Ethanol 1minute
2. Rinse in tap water
3. Harris or Gill Hematoxylin 1-3 minutes (Time vary with selection of hematoxylin solution)
4. Rinse in tap water or Scott's tap water
5. 95% Ethanol 1 minute
6. OG-6 stain for 1.5 minutes.
7. 95% Ethanol 1 minute
8. EA-50 for 2.5 minutes.
9. 95% Ethanol 1 minute, 2 changes
10. 100% Ethanol 1 minute
11. Clear in 2 changes of xylene, 2 minutes each
12. Mount with DPX

Cervical samples were compared for multiple parameters like morphology of various cells, unsatisfactory rates and sensitivity of two methods (thin prep and CS) for detection of epithelial abnormalities as per the Bethesda system (TBS) 2014. Also wherever available, the results of cervical Pap samples were correlated with follow-up cervical biopsies/resection specimens.

Five samples splited between conventional smears and liquid base solution to prepare thin prep smears-and-stained-with-P53-Immunohistochemical-stain.

The immunostaining procedure performed using an automated strainer (Leica IHC Bond Max) the primary antibody used was the mouse monoclonal antip53 antibody clone DO-7 (1:400, DAKO, Glostrup, Denmark). Then Microwave antigen retrieval performed by placing the slides in 10 mM citrate buffer (pH 6.0) in a pressure cooker (Nordic Ware), and microwaving on high power until the buffer boiled under pressure for 4 minutes. At this point microwaving stopped, and the slides incubated in the pressure cooker for another 20 minutes, removed and rinsed. The antip53 antibody DO-7 recognizes both wild type and mutant p53. Sections counterstained with light haematoxylin. P53 index assessed under a light microscope with-magnification-(10-×-and-40-×-objective).

STATISTICAL ANALYSES

Data were analyzed using the statistical package SPSS version15 Pearson Chi-square test was used to analyze the data and *P* value was calculated wherever required. *P* value of 0.05 or less was considered as statistically significant.

RESULT

"Split samples" (CS and LBC samples from the same patient). Break-up of "split samples" reported as per TBS 2014 is given (Table1).

Pap report	CS Cases	LBC Cases	Statistical analysis
Epithelial cell abnormality (ASCUS)	01	02	NS
Epithelial cell abnormality (LSIL)	01	01	NS
Unsatisfactory	4	1	P=0.0005
TV Infection	0	1	NS
Candida spores	0	2	NS

Table: 1: Comparing CS interpretation with LBC; one sample showed ASCUS in both preparations, while one sample was positive in LBC and missed in CS. Also one sample was sharing the unsatisfactory (low squamous cellularity in both SC and LBC) while Three samples were U/S in CS due to obscuring factors and were Satisfactory in LBC, TV infection missed in CS and identified in LBC, Candida spores also missed in CS and identified in LBC.

Abbreviations key:
CS: Conventional smear.
U/S: Unsatisfactory.
LBC:Liquid base cytology.

Comparison of morphological characteristics in conventional Pap smear versus liquid-based cytology

About 2% Pap samples were reported to have atypical squamous cells of undetermined significance (ASCUS) in LBC smears and 1% in CS smears of the same samples Out of a total of 100 cases while one case was missed in CS and reported as NILM. low grade squamous intraepithelial lesion (LSIL) (1%) same case identified in both CS and LBC, 4 CS smears was U/S due to hemorrhagic and excessive inflammatory smears while I LBC smear was U/S due to low squamous cellularity. (Table-1).
Low grade squamous intraepithelial lesion in LBC showed singly scattered and groups of intermediate sized squamous cells with nuclear enlargement, slight increase in nuclear: Cytoplasmic (N:C) ratio, uniformly distributed, coarsely granular nuclear chromatin and slightly irregular nuclear membranes. Koilocytosis was noted in the cases. These changes were appreciated both in CS and LBC samples, however nuclear details were much clearer in LBC smears and it was easier to appreciate koilocytosis in LBC smears. Small brightly orangeophillic dyskeratotic cells were conspicuous in LBC smears. Cases which fell short of LSIL but had changes more than reactive atypia were reported as ASCUS. Figures (2&3).

Immunohistochemical Stain
Conventional smears did not appear to confer a cytomorphological advantage and has a lower diagnostic accuracy using IHC stains. The sensitivity of Thin Prep was significantly higher than that of CS due to cellular clumps and presence of marked inflammatory cells and blood which compete other epithelial cellular elements in staining affinity in addition to the length of the smear which needs large volume of stains to cover the whole area. While the confined area of thin prep smear and homogenous cellular distribution supports the advantages of thin prep over the conventional smear when using IHC stain.

Unsatisfactory smears
There were 4/100, 4% U/S cases in CS and 1/100, 1% cases in the LBC samples. This difference is statistically significant with $P = 0.0006$. In split samples, the main cause of unsatisfactory smears cause were obscuring blood and inflammatory cells in CS in more than 80% of the smear. In LBC were due to low squamous cellularity. Figure (1).

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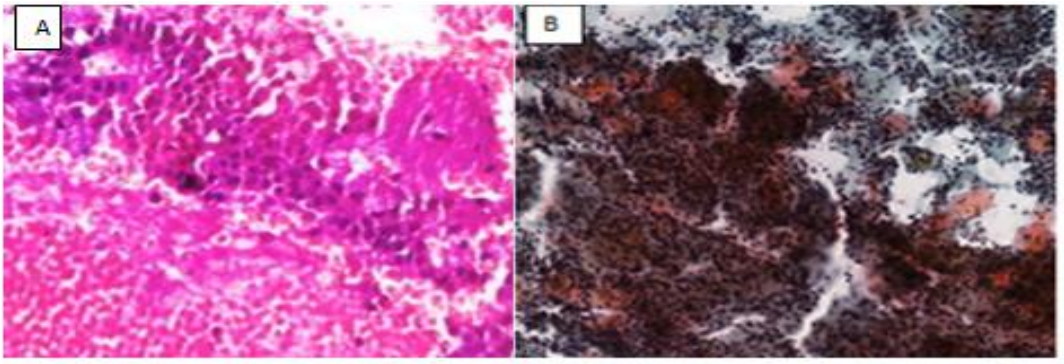

Figure 1: (A) and (B) Showing unsatisfactory conventional smear due to excessive hemorrhage and marked inflammation respectively.

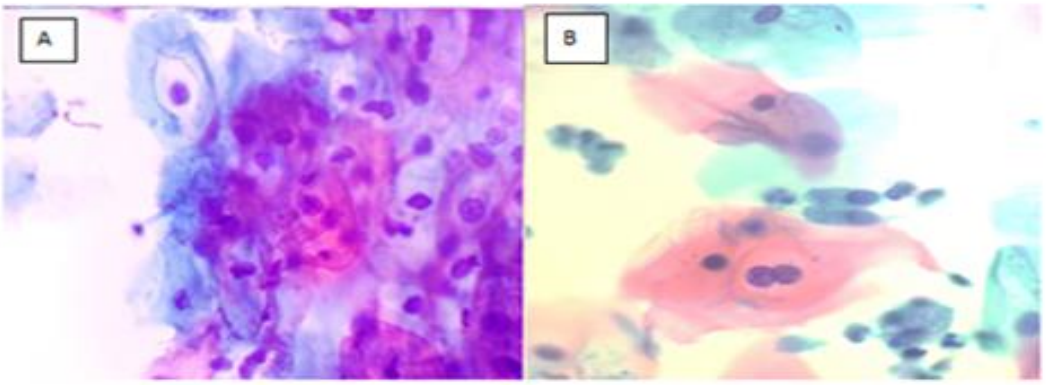

Figure 2: (A) and (B) Showing Atypical squamoius cells of undetermined significance (ASCUS) in CS and LBC respectively.

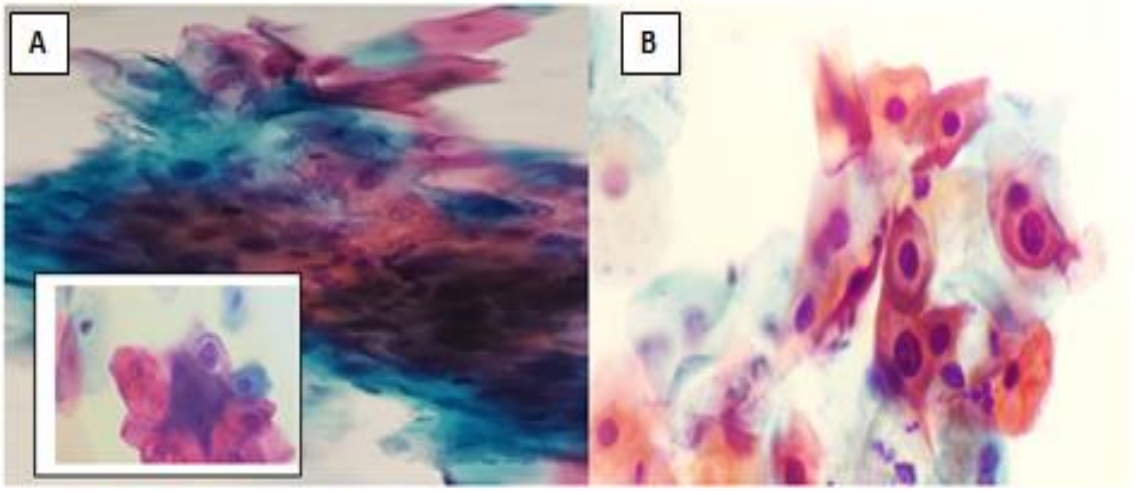

Figure 3: (A) Showing Low Grade Squamous intraepithelial lesion in Conventional smear (CS). (B): Showing Low Grade Squamous intraepithelial lesion in Liquid Base (LBC).

Infections

Trichomonas vaginalis organism and spores of candida spp were masked by squamous blocks and overlapping clumps and couldn't identified microscopically, while it was seen in LBC smears easily.

Screening time

Although this study was not designed to evaluate screening time, it was seen that the average time needed to screen a LBC slide is 2 to 3 min as compared to CS, which is at least 5 to 6 min.

DISCUSSION

Pap smear is one of the best available screening methods for early detection of cervical precancerous lesions. LBC is an alternate technique for processing the cervical sample collected. Most Western countries have switched over from CS to LBC, even though the sensitivity and specificity is almost similar in various comparison studies. The reason for this may be consistently reduced rates of U/S results on LBC, clarity of microscopy, improved sample processing, and small area to be screened. Furthermore, the potential for performing additional tests, including HPV testing on the residual sample, probably underpins the acceptability of LBC among gynecologists, colposcopists and pathologists. This study was conducted to evaluate the performance of LBC to study the differences in morphology of various lesions in CS and LBC samples.

In this study, the rate of detection for epithelial cell abnormalities was similar in both CS and LBC

except that the time frame required to screen a slide may compromise the screening skills. There are many studies which have documented similar detection rate on both types of preparations. In a direct comparison study by Taylor *et al*. of 5652 cases, CPS and LBC performance and accuracy were statistically similar. [22] . Another Japanese study with 1551 split samples, showed that the sensitivity of lesions histologically diagnosed as CIN1 or above was not significantly different between the two methods (P = 0.575-1.000) and cytologic results showed a concordance rate of 85.3% (k = 0.46) between the two methods [23]. Large meta-analyses by Arbyn *et al*. included 109 studies where positivity and/or adequacy rate was studied. In their analyses, there was no statistically difference in sensitivity and specificity between the two different methods for detection of CIN2+. [18]. However, there are other studies in the literature indicating higher detection rates of HSIL + lesions and glandular lesions in LBC [15],[21].

In the present study the CS smears which showed U/S were satisfactory in LBC preparation. The most common reason for U/S was obscuring elements i.e. polymorphs/ mucus and hemorrhage. One sample was U/S in both LBC and CS due to low squamous cellularity. Therefore, the samples with excess blood better handled by LBC. The reduction of U/S smears in LBC samples is consistent with many previous studies. [24],[25[,[26] The National Institute for Clinical Excellence in UK showed lower proportions of U/S smears from 9% in conventional cytology to 1.6% in LBC [27]. LBC leads to almost complete elimination of most causes for U/S conventional preparation, with scant cellularity remaining as the main cause for U/S LBC. [20] This can also be handled by adequate visualization of the cervical os and proper sample-taking. Infectious organisms such as Candida hyphae, TV, herpes simplex virus and actinomycetes-like organisms were seen better or more easily on the LBC samples. Candida hyphae were more easily identified in LBC as the "Shish-kebabs" of pseudo hyphae skewering the squamous cells. This effect was more pronounced in the LBC. On the other hand, Candida spores were more commonly seen present in the background on the LBC, in the present study. Therefore, the main advantage of LBC is reduction in unsatisfactory rate and availability of residual LBC sample to perform HPV DNA testing. HPV testing is of increasing importance as HPV testing is considered for incorporation into screening programs [23] as triaging low-grade abnormalities, co-testing with cytology and as a primary cervical cancer screening tool. There has been 100% conversion from CS to LBC for cervical cancer screening in the developed world. In the underdeveloped countries a low-resource setting and the cost-effectiveness of LBC as compared to CS, especially in the absence of reflex HPV testing in a majority of centers. Our study provides important context on current patterns of uptake of LBC, which is strongly dependent on a woman's age, her screening history, socioeconomic factors and ability of pay in absence of public funding. Cost-effectiveness of LBC needs to be evaluated in poor countries with benefits and harms associated with a move to LBC.

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