

Original Research Article

A study of comparison of liquid-based cytology versus conventional pap smear for evaluation of cervical cytology at a tertiary healthcare hospital

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ABSTRACT

Background: Cervical cancer is the second highest cause of cancer-related mortality in women, and the only sign of this cancer in the early stages is the loss of abnormal cells. Clinical signs of the disease appear only after cancer has reached advanced stages. Conversely, this cancer in precancerous conditions is completely curable and screening with conventional Papanicolaou (CP) has reduced the mortality by 70% but it is also associated with a significant number of false-negative cases (20-50%). In 1996, liquid-based cytology (LBC) method was developed to overcome the disadvantages of the previous method expecting to have good features such as high sensitivity, faster sample preparation, and decreased the rate of inadequate smears.

Methods: This descriptive-analytic study was conducted at the Department of Pathology and Department of Obstetrics and Gynaecology, government medical college, Datia for the period of 11 months from April 2018 to February 2019. The study includes total 80 subjects. Total No. of Pap smear examined (both LBC and CPS) are 160.

Results: There were statistically significant differences of satisfactory/unsatisfactory rate, smear cellularity, background clarity and detection of endocervical cells, in between liquid based cytology and conventional Pap smear finding ($p < 0.05$). Diagnostic efficacy i.e. sensitivity and specificity of LBC is greater than CPS for evaluation of cervical cytology.

Conclusions: Results shows, that LBC may improve the sample's quality, reduce the number of unsatisfactory smear and increases the diagnostic efficacy.

Keywords: Cervical cancer, Conventional pap smear, Liquid-based cytology, Papanicolaou

INTRODUCTION

Squamous cell carcinoma is the most common histologic subtype of cervical cancer, accounting for approximately 80% of cases.¹ Cervical cancer represents worldwide the fourth most common cancer among women.² It is the fact that most cervical carcinomas occur in women who have

never been screened or who have not been screened adequately.

Most cases of CIN remain stable or are eliminated by the host's immune system without intervention. However, a small percentage of cases progress to become cervical cancer, usually cervical squamous cell carcinoma, if left

untreated. The major cause of CIN is chronic infection of the cervix with the sexually transmitted human papillomavirus (HPV), especially the high-risk HPV types 16 or 18. HPV infection has been detected in almost all pre-neoplastic and neoplastic lesions of the cervix. HPV 16 and 18, which are known to cause at least 70% of cervical cancers.³ Other HPV types contribute to less than 5% of cases, individually. In addition to infecting squamous cells, HPVs may also infect glandular cells or neuroendocrine cells present in the cervical mucosa and cause malignant transformation, resulting adenocarcinoma, and adenosquamous and neuroendocrine carcinomas; these tumor subtypes, however, are less common since glandular and neuroendocrine cells do not support effective HPV replication.

Screening programmes for cervical cancer using the conventional paps smear technique have been in place since decades and have been successful in detecting cancers of the cervix significantly. However conventional paps smear technique has many limitations. In the conventional PAP tests, the false-negative rate for invasive carcinoma range from 16-82%. Attempting to find higher sensibility for the method, which according to a meta-analysis is 58% (varying from 11 to 99%) with specificity of 68% (varying from 14 to 97%), new techniques to collect and prepare the samples were developed so the liquid-based cytology (LBC) was introduced.

Liquid based, thin layer technology was developed to address the limitation of Pap smear. More than 5,00,000 subjects have been studied with a preponderance of data indicating a significant benefit of liquid-based, thin layer technology in the detection of cervical cancer precursor lesions and in the improvement of specimen adequacy.

The present study was undertaken to evaluate a liquid based cytology technique and to compare it sensitivity and specificity with conventional pap smear.

METHODS

This is a comparative prospective study conducted at the Department of Pathology and Department of Obstetrics and Gynaecology, government medical college, Datia for the period of 11 months from April 2018 to February 2019. Data collection was done from women attending Gynaecology OPD with complaints of symptoms related to cervical lesion and unhealthy cervix at government medical college hospital, Datia were included in study after written consent. The study includes total 80 subjects. Total No. of Pap smear examined (both LBC and CPS) are 160

Methodology of collection of data

- The samples were taken with cervix-brush (split-sample technique).

- First, a CPS was prepared and immediately alcohol-fixed.
- For LBC same brush head was suspended in preservative fluid after detachment.
- Preservative fluid, was transferred to the Cytopathology laboratory for further processing which will took place as per the prescribed protocol for the LBC equipment
- Both the slides were stained by papanicolaou technique.
- Pap smear reporting was done according to the New Bethesda System 2014 for both.

Women between 20-70 yrs of age presenting with complaints and symptom related to cervical lesion and unhealthy cervix were included in study. Exclusion criteria include the subjects below 20 yrs of age and above 70 yrs of age, patients with total hysterectomy, presence of intrauterine device, pregnant women, patients taking treatment for cervical cancer, patient already taking treatment (chemo and radio-therapy) for any type of cancer.

Statistical analysis

The statistical software namely statistical package for the social sciences (SPSS) version 22 is used for analysis of data. Sensitivity, specificity, positive predictive value and negative predictive value of pap smear were calculated with histopathology as gold standard. Chi-square test for testing the significance of percentages was used. A p value of 0.05 or less was considered significant.

RESULTS

In our study the peak age group was between 30-39 years (36.2%); most common complaints were of white discharge per vagina (WDPV) in 32 cases (40%); most common per speculum finding was cervical erosion in 60%.

In LBC 90% cases showed satisfactory smear white in CPS 75% cases showed satisfactory results. In our study presence of satisfactory smears were found to be significantly higher in LBC as compared to CPS (Figure 1). There were statistically significant differences of satisfactory/unsatisfactory rate, in between liquid based cytology and conventional Pap smear finding ($p=0.02$).

Comparison of cellularity in between conventional Pap smear and liquid based cytology shows the good cellularity of smear was seen in 63.75% cases of CPS and 27.5% cases of LBC (Figure 2). There were statistically significant differences of the smear cellularity between conventional Pap smear and liquid based cytology in terms of the cellularity finding ($p<0.0001$).

Table 1 shows comparison of clarity of background, between conventional Pap smear and liquid based

cytology. Inflammatory cells (neutrophils) were present in 50 % cases of CPS while in LBC it was 28.75%. Our study showed presence of mucin in 90% cases of CPS which was found to be higher as compared to LBC showing presence of mucin in 5% cases. Presence of hemorrhage in our study in CPS was found in 18.75% of cases while in LBC it was 3.75%. This finding that's that in our study clarity of background was found to be more for LBC in comparison of CPS. Background clarity findings were compared between conventional Pap smear and liquid based cytology in terms of background clarity, (p=0.002). Comparison of endocervical cells detection in between conventional Pap smear and liquid based cytology showed, endocervical cells were present in 46.25 % of cases in CPS while in LBC it was present in 20 % cases (Figure 3). Significantly difference was observed between conventional Pap smear and liquid based cytology in detection of endocervical cells (p<0.0001).

Table 2 Depicting comparison of microscopic findings in between conventional Pap smear and liquid based cytology. In CPS distribution of microscopic findings were as follows, normal smear 3.75 % cases, NILM 38.75% cases, epithelial abnormalities 35% cases. Whereas in LBC normal smear were found 7.5% cases, NILM 46.25 % cases, and epithelial abnormalities cases 41.25% cases.

Table 3 Shows comparison of distribution of cases in NILM category between conventional Pap smear and liquid based cytology. CPS technique depicting that out of total 31 cases of NILM, maximum no of cases in 74.19% were in BRCC followed by Trichomonas vaginalis in 6.4% cases, *Candida albicans* and bacterial

vaginosis in (6.4%) cases. In present study in LBC technique out of 37 cases of NILM category maximum number of cases i.e. 54.05% cases were of BRCC.

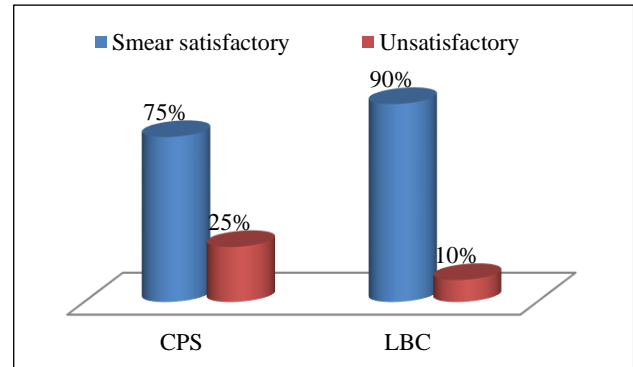


Figure 1: Satisfactory smears in LBC as compared to CPS.

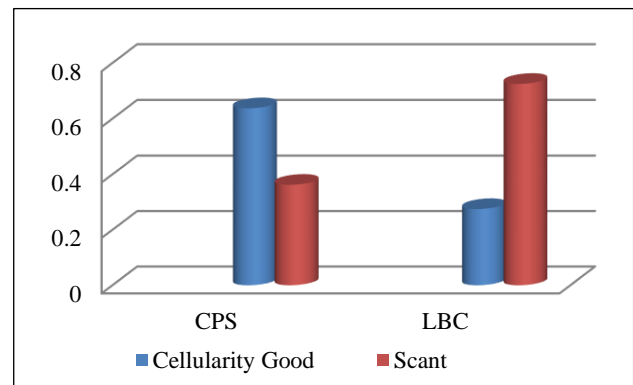


Figure 2: Comparison of cellularity in between conventional Pap smear and liquid based cytology.

Table 1: Comparison of clarity of background, between conventional pap smear and liquid based cytology.

			Diagnostics techniques		Total	P-value
			Conventional pap smear	Liquid based cytology		
Inflammation	Present	n	40	23	63	0.002
		%	50%	28.75%	39.38%	
	Absent	n	40	57	97	
		%	50%	71.25%	60.62%	
Total	n	80	80	160		
	%	100%	100%	100%		
Mucin	Present	n	72	04	78	<0.001
		%	90%	5%	47.5%	
	Absent	n	08	76	82	
		%	10%	93.75%	52.5 %	
Total	n	80	80	160		
	%	100%	100%	100%		
Haemorrhage	Present	n	15	3	18	0.01
		%	18.75%	3.75%	11.25%	
	Absent	n	65	77	142	
		%	81.25%	95.25%	88.75%	
Total	n	80	80	160		
	%	100%	100%	100%		

Table 2: Depicting comparison of microscopic findings in between conventional Pap smear and liquid based cytology.

		Diagnostics techniques		Total	P-value
		Conventional pap smear	Liquid based cytology		
Normal smear	n	03	06	9	0.02
	%	3.75%	7.5%	5.6 %	
NILM	n	31	37	68	
	%	38.75%	46.25%	42.25%	
Total	n	80	80	160	
	%	100%	100%	100%	

Table 3: Comparison of distribution of cases in NILM category between conventional Pap smear and liquid based cytology.

		Diagnostics techniques	
		Conventional pap smear	Liquid based cytology
BRCC benign reactive cellular changes of inflammation	N	23	20
	%	74.19%	54.05%
Trichomonas vaginalis	N	02	06
	%	6.4%	16.21%
Candida albicans	N	02	05
	%	6.4%	13.51%
Bacterial vaginosis	N	02	04
	%	6.4%	10.81%
Atrophic smear	N	02	02
	%	6.4%	5.4%
Total	N	31	37
	%	100%	100%

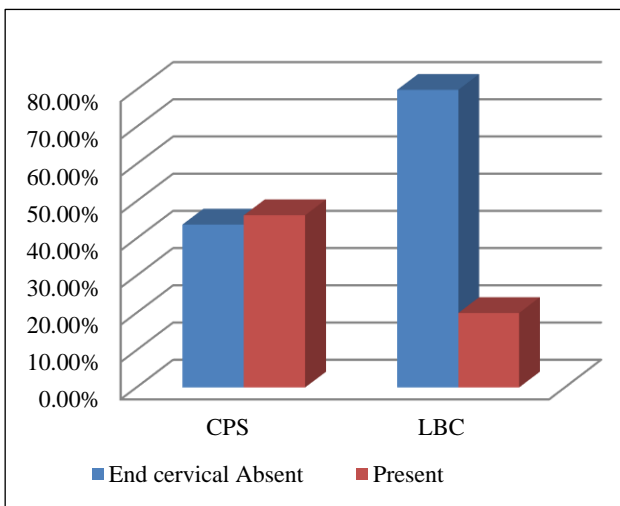


Figure 3: Comparison of endocervical cells detection in between conventional Pap smear and liquid based cytology.

Microscopic findings were compared between conventional Pap smear and liquid based cytology using

chi square test. No significant difference was observed between conventional Pap smear and liquid based cytology in microscopic findings (p=NS).

Table 5 shows histopathological finding observed in our study. Most common findings were CIN I (37.5%) followed by CIN III (20%), Chronic cervicitis (17.5%), and CIN II (15%) and SCC (7.5%) (Figure 5).

Comparison of LBC with HPE findings in low grade squamous intraepithelial lesion (LSIL) shows sensitivity: 93.3, specificity: 100%, positive predictive value-100 and negative predictive value-96.1%. Comparison of CPS with HPE findings in low grade squamous intraepithelial lesion (LSIL) show sensitivity-80%, specificity: 92%, positive predictive value-85.71%, negative predictive value-88.4%.

Comparison of LBC with HPE finding in high grade squamous intraepithelial lesion (HSIL) shows sensitivity: 50%, specificity: 100%, positive predictive value-100%, Negative predictive value-78.78%. Comparison of CPS with HPE finding in high grade squamous intraepithelial lesion (HSIL) shows sensitivity-42.8%, specificity:

92.30%, positive predictive value-75%, negative predictive value-80%.

Table 4: Comparison of distribution of epithelial abnormalities between conventional pap smear and liquid based cytology.

Microscopic findings	Diagnostics techniques		P-value
	Conventional pap smear	Liquid based cytology	
ASCUS	N 07 % 8.75%	09 10%	0.99
LSIL	N 12 % 15%	14 17.5%	
HSIL	N 06 % 7.5%	07 8.75%	
SCC	N 02 % 2.5%	02 2.5%	
AGC-NOS	N 01 % 1.25%	01 1.25%	
Total	N 28 % 35%	33 41.25%	

Table 5: Histopathological finding observed in our study.

Histopathological findings	Frequency	Percent
Chronic cervicitis	07	17.5 %
CIN I	15	37.5 %
CIN II	06	15 %
CIN III	08	20 %
Squamous cell carcinoma	03	7.5 %
Inadequate biopsy	1	2.5 %
Total	40	100%

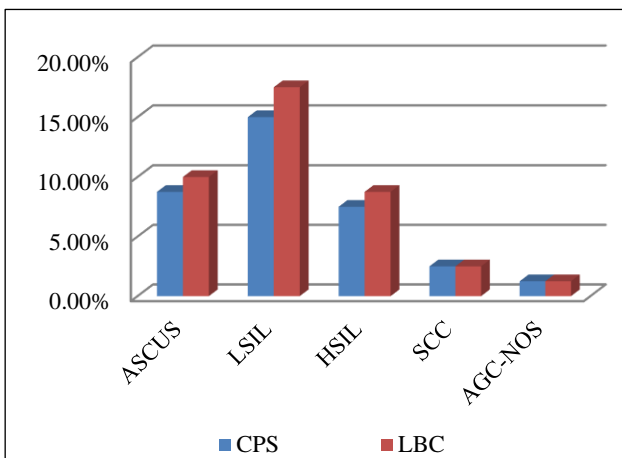


Figure 4: Comparison of distribution of epithelial abnormalities between conventional pap smear and liquid based cytology.

Comparison of LBC with HPC finding Invasive (SSC) show sensitivity: 66.66%, specificity: 100%, positive

predictive value-100%, negative predictive value-97.3%. Comparison of CPS with HPC findings in Invasive (SSC) shows sensitivity-66.66%, specificity: 100%, positive predictive value-100%, negative predictive value-97.3%.

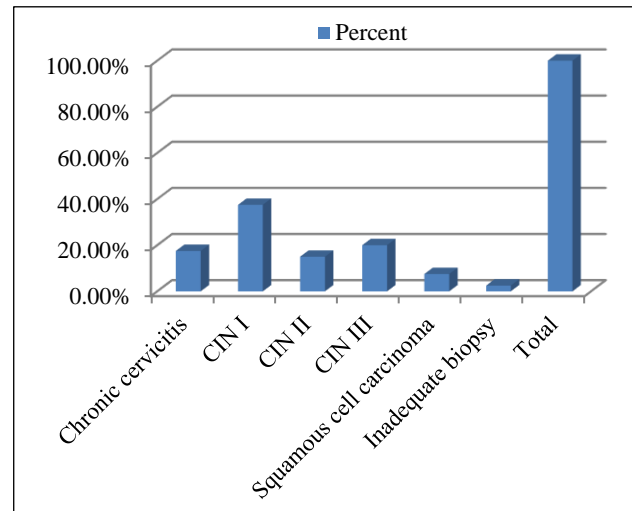


Figure 5: Histopathological finding observed in our study.

Table 6: Diagnostic efficacy of CPS and LBC for evolution of cervical cytology.

	LSIL		HSIL		SCC	
	CPS	LBC	CPS	LBC	CPS	LBC
Sensitivity %	80	93.3	42.8	50	66.6	66.6
Specificity %	92	100	92.3	100	100	100
PPV%	85.1	100	75	100	100	100
NPV%	87.4	96.1	80	78.7	97.3	97.3

Table 6 shows diagnostic efficacy of CPS and LBC for evaluation of cervical cytology when comparison done with histopathological examination. Sensitivity of CPS for detection of LSIL lesion were (80%), for HSIL lesion (42.8%), for SSC (66.6%) while sensitivity of LBC were (93.3%), (50%) and (66.6%) for LSIL, HSIL, SCC lesion respectively. Specificity of LBC were (100%) for all category of epithelial abnormalities, while in CPS it was (92%) for LSIL, (92.3%) for HSIL % (100%) for SCC. PPV of CPS for LSIL lesion were (85.1%) for HSIL lesion (75%) for SCC (100%), whereas PPV of LBC was (100%) for all category of epithelial abnormalities. NPV of CPS for LSIL lesion (87.4%), for HSIL lesion (80%) for SCC (97.3%), while for LBC it was (96.1%) for LSIL, (78.7%) for HSIL (97.3%) for SCC.

DISCUSSION

In the present study maximum numbers of cases were noted between 30-39 years. Similar finding of maximum number of patients presenting to age group of 30 to 35 years were observed by Ranjana H et al.⁴ Whereas Afsan

N et al, observed maximum no of cases in age group was 21-40 years and 25-44 years respectively.⁵

In present study most common presenting complaint was white discharge per vagina (40%). Similar finding were observed by N Afsan et al, they also found that most common presenting complaints in their study was white discharge per vagina, in (42.5%) cases.⁵ Kenneth and Yao, have emphasized the significance of vaginal discharge and its association with neoplastic changes in the cervix.⁶ These finding was also observed in our study, i.e. patients presenting with complaints of white discharge were associated with neoplastic lesions in cervix. Our finding is in discordance with the study of Karimi-Zarchi M et al who reported most common presenting complaint was post-menopausal bleeding in 30.7%.⁷

The present study, the percentage of inflammatory results with the CPS method was 50% vs 28.7% with LBC. Inflammatory findings showed significant difference between conventional Pap smear and liquid based cytology ($p=0.002$). This is in concordance of the study of Costa MOLP et al, they observed that inflammatory results with the CPS method was 58.47% vs 46.4 7% with LBC and significant difference in inflammatory background between CPS and LBC with p value <0.01 .⁸

Sharma J et al, reported that in their study neutrophils showed more clumping in LBC smears while they were scattered in CPS.⁹ Clumping of neutrophils in LBC was said to be due to tendency of LBC preservative fluid to stick to the inflammatory exudates forming clumps. This also explains the cleaner background seen in LBC smears. Despite the cleaner background and reduced neutrophils, usually inflammation is not missed out in LBC because as noted by other authors, the neutrophils (although reduced) are clearly seen in LBC 44, similar findings were found in our study.

CPS showed presence of mucin in background in 90% cases while in LBC it was 5% case in our study, which is similar to study done by, Sulochana S et al who also observed that mucin was present in 100% case in CPS while it was present in 1.8% case in LBC.¹⁰

Sulochana S et al, found in their study that for sufficient light to penetrate the slide, and illuminate the cells clearly it is essential that the background of the cells in the smear is clear and free of mucous and other artifacts.¹⁰ In LBC, mucin and other artifacts were removed in the procedure of processing. Hence only the representative cells were present and the background was completely free of mucin and other obscuring artifacts.

This ensured that the cells were clearly seen against a bright background. In the conventional smear, mucous along with the required cells were smeared on the slide. In addition, drying artifacts were also present. This obscured the background and also the cellular

morphology resulting in poor illumination of the smears, thereby making it difficult to study the smear. Therefore, using LBC, it was possible to overcome these shortcomings of a conventional smear, 4 we also observed similar result in our study.

In our study hemorrhage was present in the background in 18.75% case in CPS as compared to 3.75% case in LBC. These finding depicted that by using LBC hemorrhage in background was reduced.

This is in concordance with the study of Sharma J et al, in their study hemorrhagic background and RBCs were encountered more frequently in CPS (11%) as compared to LBC (3%) with a statistically significant p value of <0.05 .⁹ A lesser number of RBCs in LBC makes screening easier.

In our study we observed that endocervical cells were present in 46.25% cases in CPS while in LBC it was 20% which is statistically significant with p value of <0.0001 .

This is in concordance of the studies of Kirschner B et al.¹¹ They also observed statistically significant difference in endocervical cells detection in between conventional pap smear and liquid based cytology.

This finding in our study in consistence with other studies is justified because smears were collected by split sample technique. Firstly, slide for CPS was prepared, then for LBC same brush head was suspended in preservative fluid after detachment so that this technique would provide more transfer of such cells to the slide.

Our finding showed discordance with the studies of Sharma J et al, who reported that with regard to the presence of endocervical cells/metaplastic squamous cells, LBC gave better results.⁹ Their method of collection of sample was not split sample technique, they used to collect two samples in one sitting.

In our study we found that % of NILM in LBC was more (46.25%) than CPS (38.75%), this finding is in concordance with study done by Ranjana H et al who also observed that % of NILM was more in LBC (80.6%) as compared to CPS (72.6%).⁴

In the present study detection of LSIL increased from 15 % to 17.5 % with LBC than with CPS and similarly detection of HSIL increased from 7.5% to 8.75% with LBC than with CPS, these findings were statistically not significant ($p=0.99$).

Similar results were found by studies done by, Ranjana H et al, Singh VB et al.^{4,12} They also observed that detection of epithelial cell abnormalities between the two methods LBC vs CPS were significantly not different.

In present study in CPS out of 31 NILM cases, two cases of trichomonas, bacterial vaginosis and Candida each

(Table 6), while in LBC out of 37 NILM cases six case of trichomonas, five cases of *candida* and four cases of bacterial vaginosis were detected.

In our study detection of infectious organism was higher (39%) in LBC as compared to (23.4%) In CPS. This finding is supported by study done by Afsan et al.⁵ In their study infectious agents were detected in 14 (8.7%) cases on Pap spin and in 5 (3.1%) cases on conventional Pap smear, *Candida* was the commonest infectious agent in 7 (4.3%) cases, followed by *Trichomonas vaginalis* in (4) cases, out of which 6 cases (85.8%) were detected on Pap spin smears .

The microscopic details of infectious agents were enhanced on LBC with candida, coccobacilli and trichomonas being readily detected. These organism were seen better or more easily on the LBC sample. *Candida hyphae* were more easily identified in LBC as the Shish-kebab of pseudohyphae skewering the squamous cells. This effect is more pronounced in the LBC.

This finding is in discordance with study of Jyotsna Sharma et al, who observed that CPS (55%) is more effective for detection of infective organism than LBC (37%).⁹

In present study Sensitivity of LBC (93.3%), for detection of a histological proven lesions (CIN I LSIL) was significantly higher than CPS (80%). similar observation was reported by Beerman H et al, they observed that liquid based cytology had a significantly higher sensitivity than the conventional Pap to detect LSIL+ lesions.¹³ Arbyn et al, reported similar sensitivity and specificity of two methods.¹⁴ This is in discordance with our study.

Our study also showed significantly higher specificity of LBC (100%) then CPS (92%) when using LSIL as cytological cutoffs.

Our findings showed discordance with Beerman H et al and Arbyn et al they reported similar specificity between the two methods.^{13,14}

In present study, sensitivity of LBC (50%) was significantly higher than CPS (42.8%) for detection of histologically proven HSIL lesions and specificity was also significantly higher in LBC (100%) as compared to CPS 96.3%).

Studies done by Afsan N et al and Beerman H et al found that in their studies sensitivity of LBC were higher than CPS, this is consistent with our study.^{5,13}

Large meta-analyses by Arbyn et al, included 109 studies where positivity and/ or adequacy rate was studied.¹⁴ In their analyses, there was no statistically difference in sensitivity and specificity between the two different

methods for detection of CIN2+, these findings are in discordance with our study.

CONCLUSION

LBC shows an almost complete elimination of most causes for unsatisfactory CPS, with scant cellularity remaining as the sole cause for unsatisfactory LBC. Therefore, LBC can be considered superior to conventional smear with respect to adequacy of smear, clarity of background, detection of infective organisms and increased sensitivity and specificity. The study confirmed previous reports of decreased numbers of unsatisfactory samples with Liquid based cytology and showed an increased sensitivity when taking LSIL and HSIL as cytological cut offs level.

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