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Original Article

Liquid-based Cytology versus Conventional Cytology for Evaluation of Cervical Cytology in a Tertiary Care Center of Chhattisgarh

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Abstract

Introduction: Cervical cancer is the second most common type of cancer among women's according to the World Health Organization. Worldwide screening programs for cervical cancer-based on the Papanicolaou smear have contributed to the decrease in incidence and mortality of cervical cancer.

Objective: To compare the adequacy of smears and diagnostic difference of conventional conventional pap smear [CPS] versus liquid-based cytology (LBC).

Materials and Methods: A prospective observational study was conducted in a tertiary care referral institute in 80 consecutive cervical "split samples" over a period of 1-year. Samples were taken with cervex-brush, first a CPS was prepared then for LBC same brush head was suspended in preservative fluid after detachment and processed by SurePath™ LBC.

Results: There were 92.5% satisfactory smears in LBC while in CPS it was 78.8%, with statistically significant difference P = 0.02. Endocervical cells were present in 57.5% of cases in CPS, while in LBC, it was present in 18.8%. Clarity of background was significantly increased with LBC. Infectious organisms were better detected in LBC. Sensitivity for detection of low-grade squamous intraepithelial lesion (LSIL) (78.6%) and for high-grade squamous intraepithelial lesion (HSIL) (72.7%) was higher in LBC as compare to 71.4% and 63.6% in CPS, respectively. Specificity was also higher in LBC 100% for both LSIL and HSIL, whereas in CPS, it was 95.8% for LSIL and 96.3% for HSIL.

Conclusion: In the present study, adequacy rate for LBC significantly increased, and the performance of LBC technique was better than CPS.

Key words: Liquid-based cytology, Conventional pap smear, Spilt sample, Adequacy rate, SurePath™

INTRODUCTION

Cervical cancer is the second most common type of cancer among women's according to the World Health Organization.[1] Worldwide screening programs for cervical cancer-based on the Papanicolaou smear have contributed to the decrease in incidence and mortality of cervical cancer.[2] One of the major advance in cervical cancer screening was the pap test. The second major advance was liquid-based cytology (LBC).[3] Screening programs for cervical cancer using the conventional pap smear (CPS) technique have been in place for decades and have been successful in detecting cancers of the cervix significantly. However, CPS technique has many limitations.[4]

The LBC corresponds to a sampling where cells are put in suspension in a conversation liquid.[5]

LBC is proposed to have many benefits over CPS such as less number of unsatisfactory (U/S) smears, more representative transfer of cells from collecting device, evenly distributed cellular material, the choice of using

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residual cellular material for human papillomavirus testing.[6]

Diagnostic accuracy of LBC when compared to CPS is a matter of great debate. Several studies have shown increased sensitivity of LBC over CPS, whereas others showing decreased or equal sensitivity and specificity.[6]

So with this background the present study was undertaken, to study the differences in diagnosis between conventional and LBC methods in cervical cytology in our setting.

MATERIALS AND METHODS

Prospective cross-sectional, observational hospital-based study was conducted in the Department of Pathology and Department of Obstetrics and Gynaecology, Pt. J.N.M. Medical College and Dr. B.R.A.M. Hospital Raipur Chhattisgarh, India, from February 2015 to January 2016.

Women attending gynecology OPD with complaints of symptoms related to cervical lesion and unhealthy cervix at Dr. B.R.A.M. Hospital, Raipur, were included in the study after written consent. A detailed clinical history and examination of the patient was taken and all details of clinical examination were noted as per pro forma.

Inclusion Criteria

Women between 20 and 65 years of age presenting with complaints and symptoms related to cervical lesion and unhealthy cervix.

Exclusion Criteria

- Women <20 years of age and >65 years of age
- Patients with total hysterectomy
- Presence of intrauterine device
- Pregnant women
- Patients taking treatment (chemo and radiotherapy) for any type of cancer.

The samples (pap smears) were taken with cervex-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 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. Cervical samples were compared for multiple parameters like unsatisfactory rates, diagnostic difference in various parameters for two methods.

RESULTS

This study was conducted in 80 patients having uterine cervix lesions, from those attending the outpatient Department of Obstetrics and Gynecology in Pt. J.N.M. Medical College and associated Dr. B.R.A.M. Hospital Raipur during the 1-year period from February 2015 to January 2016. The results of LBC and CPS were compared and analyzed. The peak age group of patients for cervical lesion was between 31 and 40 years (36.2%).

Figure 1 shows the most common presenting complaints was white discharge per vagina in 33 cases (41.25%).

Table 1 shows most common per speculum finding was cervical erosion in 58.75%.

Table 2 shows in LBC 92.5% cases showed satisfactory smears while in CPS 78.8% cases showed satisfactory results ($P = 0.02$).

Figure 2 shows clarity of background was more in LBC as compared to CPS.

Figure 5 shows comparison of Photomicrograph finding of CPS & LBC, liquid-based cytology smear showing clear background, while conventional pap smear showing presence of marked inflammatory cells which obscured the smear

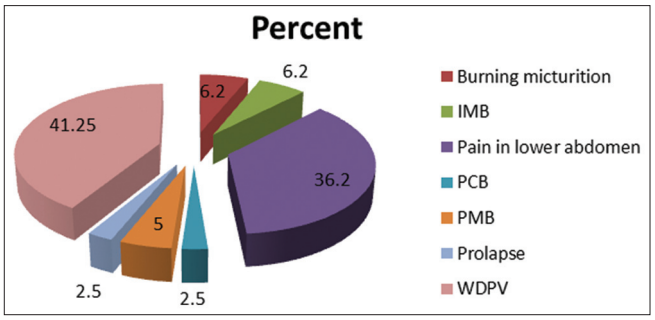

Figure 1: Distribution of cases in relation to presenting complaints (*n* = 80)

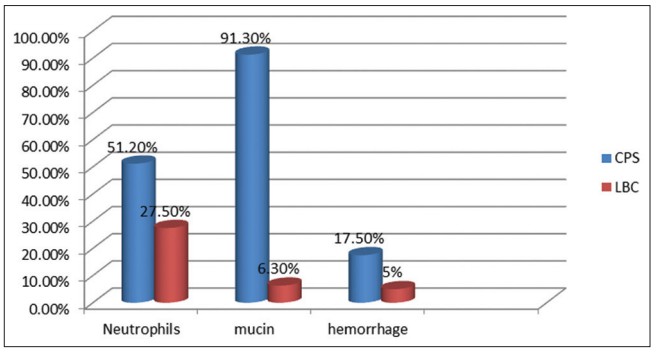

Figure 2: Comparison of clarity of background, between conventional pap smear and liquid-based cytology

Table 3 shows detection of endocervical cells significantly increased with CPS ($P < 0.0001$).

Figure 3 shows in CPS distribution of microscopic finding were as follows, normal smear (5%) cases, negative for intraepithelial lesion or malignancy (NILM) (37.5%) cases, epithelial abnormalities (36.25%) cases. Whereas in LBC normal smear were found (7.5%) cases, NILM (45%) cases, and epithelial abnormalities (40%) cases.

Table 4 shows in LBC no of cases of atypical squamous cells of undetermined significance were 11.2%, while in CPS, it was 10% of cases. Our study shows that in LBC (13.7%) cases of low-grade squamous intraepithelial lesion (LSIL) were reported while 12.5% of cases were reported in CPS. In high-grade squamous intraepithelial lesion (HSIL) category, 10% of cases reported in LBC; while in CPS, it was 8.7% of cases. Detection of cases in squamous cell carcinoma (SCC) and AGS-NOS category were same in LBC and CPS, 3.75% and 1.25%, respectively.

Table 1: Distribution of cases according to per speculum finding

Perspeculum finding	Frequency (%)
Cervical erosion	47 (58.75)
Cervical growth	3 (3.75)
Cervical hypertrophy	27 (33.8)
Cervical polyp	1 (1.2)
Procedentia	2 (2.5)
Total	80 (100.0)

Table 2: Comparison of satisfactory/unsatisfactory rate, in between conventional pap smear and liquid-based cytology

Detection rate	Diagnostic technique *n* (%)			*P* value
	Conventional pap smear	Liquid-based cytology	Total	
Smear				
Satisfactory	63 (78.8)	74 (92.5)	137 (85.6)	0.02
Unsatisfactory	17 (21.2)	6 (7.5)	23 (14.4)	
Total	80 (100.0)	80 (100.0)	160 (100.0)	

Table 3: Comparison of endocervical cells detection in between conventional pap smear and liquid-based cytology

Endocervical cells	Diagnostic technique *n* (%)			*P* value
	Conventional pap smear	Liquid-based cytology	Total	
Absent	34 (42.5)	65 (81.3)	99 (61.8)	<0.0001
Present	46 (57.5)	15 (18.8)	61 (38.1)	
Total	80 (100.0)	80 (100.0)	160 (100.0)	

Figure 7 shows comparison of Photomicrograph finding of CPS & LBC pap smear, of a case of low-grade squamous intraepithelial lesion showing mature squamous cells with enlarged nuclei with variable chromatin and nuclear membranes,

Figure 4 shows histopathological finding observed in our study. The most common finding was cervical intraepithelial neoplasia (CIN) I (36.8%) followed by CIN III (18.4%), chronic cervicitis (15.7%), and CIN II (10.5%), and SCC (13.1%).

Table 5 shows diagnostic efficacy of CPS and LBC for evaluation of cervical cytology. Sensitivity of CPS for detection of LSIL lesions was 71.4% for HSIL lesions (63.6%). While sensitivity of LBC was 78.6%, 72.7% for LSIL, HSIL, lesions respectively. Specificity of LBC was 100% for all categories of epithelial abnormalities; while in CPS, it was 95.8% for LSIL, 96.3% for HSIL. Positive predictive value (PPV) of CPS for LSIL lesions were 90.9%, for HSIL lesions (87.5%), whereas PPV of LBC was 100% for both categories of epithelial abnormalities. Negative predictive value of CPS for LSIL lesions (85.1%) and for HSIL lesions (86.6%); while for LBC, it was 88.9% for LSIL, 90% for HSIL.

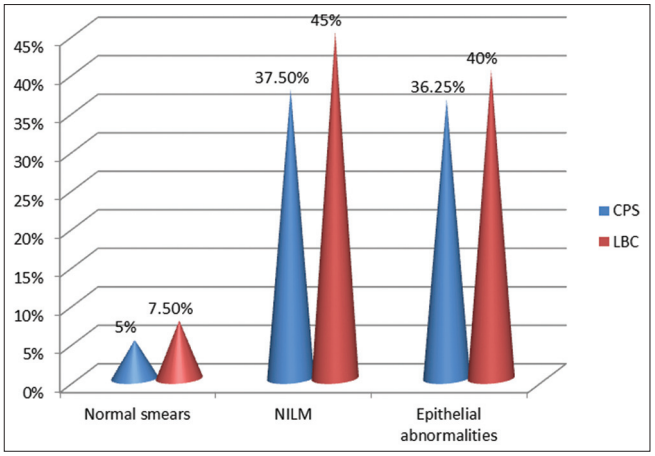

Figure 3: Comparison of microscopic findings in between conventional pap smear and liquid-based cytology

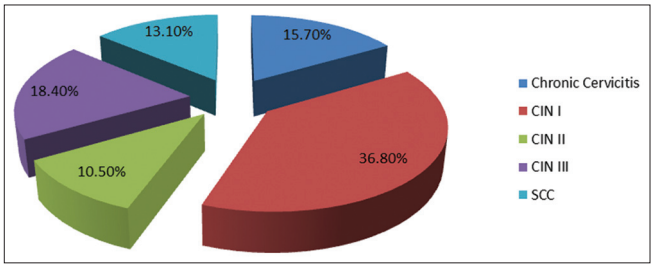

Figure 4: Histopathological finding (*n* = 38)

DISCUSSION

In the present study, a maximum number of cases were noted between 31 and 40 years. Similar finding of a maximum number of patients presenting to the age group of 30-35 years were observed by Siebers *et al.* (2009)[7] and Ranjana and Sadhna (2016).[4]

Whereas Afsan *et al.* (2007)[8] and Longatto-Filho (2015)[9] observed maximum no of cases in age group was 21-40 years and 25-44 years, respectively.

In the present study, the most common presenting complaint was white discharge per vagina (41.25%). Similar finding was observed by Afsan *et al.* (2007)[8] and Neelima (2012).[10]

Table 6 shows that in our study presence of satisfactory smears were found to be significantly higher in LBC as compared to CPS. In LBC it was (92.5%) while in CPS it was (78.8%).

Our finding was in concordance with the studies of Bergeron (2001),[5] Afsan *et al.* (2007),[8] Strander *et al.* (2007),[11] Beerman *et al.* (2009),[2] Singh *et al.* (2015),[6] and Costa (2015).[12] They also reported an increased number of satisfactory cases on LBC than conventional smears, which was statistically significant.

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

Microscopic finding	Diagnostic technique *n* (%)		*P* value
	Conventional pap smear	Liquid-based cytology	
Microscopic findings			
ASCUS	8 (10)	9 (11.2)	0.99
LSIL	10 (12.5)	11 (13.7)	
HSIL	7 (8.7)	08 (10)	
SCC	3 (3.75)	03 (3.75)	
AGC-NOS	1 (1.25)	01 (1.25)	
Total	29 (36.25)	32 (40)	

LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, SCC: Squamous cell carcinoma

Table 5: Diagnostic efficacy of CPS and LBC for evaluation of cervical cytology

Diagnostic parameters	LSIL		HSIL	
	CPS	LBC	CPS	LBC
Sensitivity (%)	71.4	78.6	63.6	72.7
Specificity (%)	95.8	100	96.3	100
PPV (%)	90.9	100	87.5	100
NPV (%)	85.1	88.9	86.6	90

LSIL: Low-grade squamous intraepithelial lesion, HSIL: High-grade squamous intraepithelial lesion, CPS: Conventional pap smear, LBC: Liquid-based cytology, PPV: Positive predictive value, NPV: Negative predictive value

Afsan *et al.* (2007)[8] observed that all drying artefact and cytolysis is almost absent or minimal with LBC because of immersion of cells into the liquid fixative and specimen adequacy was greatly improved due to the absence of limiting factors such as blood, mucus, and inflammatory cells. Only conventional smears were unsatisfactory due to thick smear, which was not a problem with LBC due to even distribution of cells. These findings are in concordance with our study.

Our finding is in discordance with the studies of Stabile *et al.* (2015),[13] Sharma *et al.* (2015).[14] They reported that there was no statistically significant difference between adequacy rate of CPS and LBC.

In the present study, the percentage of background inflammation significantly reduced with LBC (27.5%) compared to CPS (51.2%) with *P* value (0.002). This is in

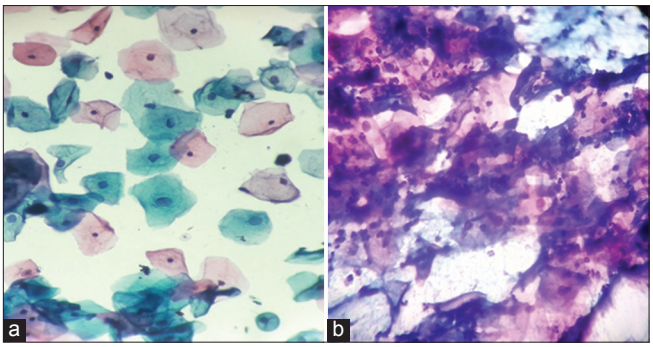

Figure 5: Photomicrograph of liquid-based cytology smear showing clear background, while conventional pap smear showing presence of marked inflammatory cells which obscured the smear (PAP, ×400). (a) Liquid based cytology (satisfactory), corresponding. (b) Conventional pap smear (unsatisfactory)

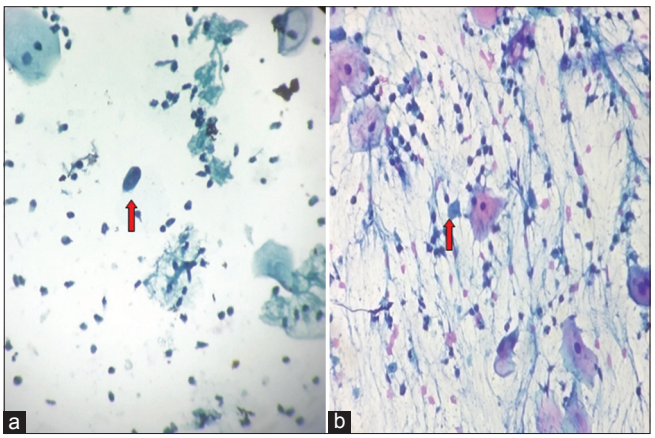

Figure 6: Photomicrograph of pap smear of a case of trichomonas vaginalis showing rounded or ovoid cyanophilic organism, cytoplasmic granules and outline of a nucleus can be discerned, the organism may be better visualized on liquid based preparations (PAP, ×400). (a) Liquid based cytology, corresponding (b) conventional pap smear of same patient

Table 6: Comparison of detection of satisfactory smears in between conventional and LBC reported by different authors

Authors	Years	Satisfactory smears in CPS (%)	Satisfactory smears in LBC (%)	*P* value
Bergeron *et al*.[5]	2001	88.4	99.2	<0.001
Afsan *et al*.[8]	2007	31.9	83.1	-
Strander *et al*.[11]	2007	99.7	99.3	0.002
Beerman *et al*.[2]	2009	99.11	99.87	<0.0001
Singh *et al*.[6]	2015	95.7	98.3	0.0006
Costa *et al*.[12]	2015	98.29	95.62	0.02
Stabile *et al*.[13]	2015	98	99	NS
Sharma *et al*.[14]	2016	92	93	NS
Present study	2016	78.8	92.5	0.02

CPS: Conventional pap smear, LBC: Liquid-based cytology

Table 7: Comparison of endocervical cells detection in between conventional pap smear and LBC reported by different authors

Authors	Year	CPS (%)	LBC (%)	*P* value	Sample technique
Bergeron *et al*.[5]	2001	6.2	6.8	-	Not split sample
Kirschner *et al*.[16]	2006	-	-	<0.005	-
Beerman *et al*.[2]	2009	86.1	89.01	<0.0001	Not split sample
Sueli Aparecida Batisa Stabile[13]	2015	93	84	Statistically significant	Split sample
Longatto-Filho *et al*.[9]	2015	58	56.7	No difference	Not split sample
Sharma *et al*.[14]	2016	16	31	0.008	Not split sample
Present study	2016	57.5	18.8	<0.0001	Split sample

CPS: Conventional pap smear, LBC: Liquid-based cytology

Table 8: Comparison of sensitivity and specificity of CPS and LBC methods (by taking LSIL as a cytology cut-offs), reported by different authors

Authors	Years	Sensitivity (%)		Specificity (%)	
		CPS	LBC	CPS	LBC
Longatto Filho *et al*.[21]	2005	49.8	70	88.2	75.4
Beerman *et al*.[2]	2009	92.04	96.24	98.2	97.8
Arbyn *et al*.[22]	2008	75.6	79.1	81.2	78.8
Present study	2016	71.4	78.6	95.8	100

CPS: Conventional pap smear, LBC: Liquid-based cytology, LSIL: Low-grade squamous intraepithelial lesion

Table 9: Comparison of sensitivity and specificity of CPS and LBC methods (by taking HSIL as cytology cut-offs), reported by different authors

Authors	Years	Sensitivity (%)		Specificity (%)	
		CPS	LBC	CPS	LBC
Bergeron C *et al*.[5]	2001	82	86	40	43
Longatto Filho *et al*.[21]	2005	72.8	91.3	85.2	70.9
Taylor *et al*.[23]	2006	69.1	60.3	94.5	94.1
Afsan *et al*.[8]	2007	53.7	97.6	50	50
Zhu *et al*.[24]	2007	47	66	-	-
Beerman *et al*.[2]	2008	93.46	97.19	98.2	97.8
Arbyn *et al*.[22]	2008	55.2	57.1	96.7	97
Present study	2016	63.6	72.7	96.3	100

CPS: Conventional pap smear, LBC: Liquid-based cytology, HSIL: High-grade squamous intraepithelial lesion

concordance of the study of Costa (2015)[12] and Sharma *et al.* (2016);[14] they also observed reduction of background inflammation with LBC.

Our study found that mucin was present in the background in CPS was higher as compared to LBC, which is similar to study done by Sulochana *et al.* (2014).[15]

In our study, hemorrhage was present in the background in 17.5% case in CPS as compared to 5% case in LBC. These finding depicted that by using LBC hemorrhage in background was reduced. This is in concordance with the study of Sharma *et al.* (2015)[14] (Table 7).

In our study, we observed that endocervical cells were present in 57.5% cases in CPS while in LBC it was 18.8% which is statistically significant with $P < 0.0001$.

This is in concordance of the studies of Kirschner *et al.* (2006)[16] and Stabile *et al.* (2015).[13] They also observed statistically significant difference in endocervical cells detection in between CPS and LBC.

This finding in our study in consistence with other studies is justified because smears were collected by split sample technique. First, slide for CPS was prepared, then for LBC same brush head was suspended in the preservative fluid after detachment so that this technique would provide more transfer of such cells to the slide.[13]

Endocervical cells are less adequately transferred to the vial because they are more likely to be trapped in endocervical mucus that is held by the collection hairs of the cervex-brush and thus are less easily dispersed in the

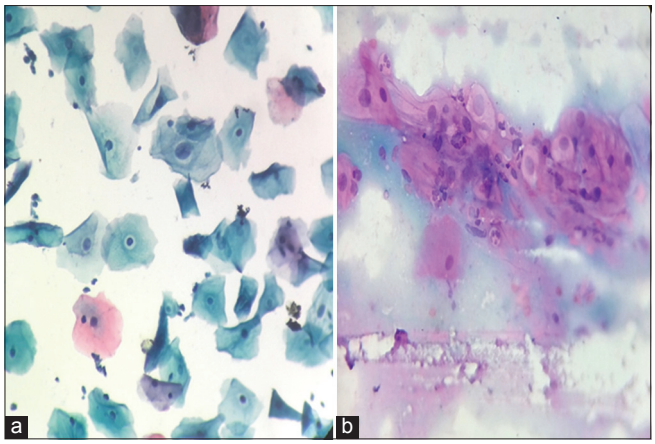

Figure 7: Photomicrograph of pap smear of a case of low-grade squamous intraepithelial lesion showing mature squamous cells with enlarged nuclei with variable chromatin and nuclear membranes, in liquid-based cytology, nuclear hyperchromasia is less evident (PAP, ×400). (a) Liquid based cytology (b) conventional pap smear

PreservCyt fixative. Furthermore, trapping of cylindrical cells in endocervical mucus during the processing of the LBC sample could potentially affect the recovery of these specific cells.[17]

Our finding showed discordance with the studies of Beerman *et al.* (2009),[2] Sharma *et al.* (2016),[14] who reported that with regard to the presence of endocervical cells/metaplastic squamous cells, LBC gave better results.

Their method of collection of the sample was not split sample technique; they used to collect two samples in one sitting. Initially, a direct pap smear was made (CPS) using an Ayre spatula, for conventional Pap staining, The second sample was collected in a vial for LBC using the detachable endocervical brush. CPS is usually associated with some loss of cells in the collecting device while LBC is likely to be more representative due to direct transfer of the entire collecting device for the preservation and homogenization of the sample during processing.

Detection of epithelial cell abnormalities between the two methods LBC versus CPS was significantly not different. Similar result were found by studies done by Siebers *et al.* (2008),[18] Beerman *et al.* (2009),[2] Am Fam Physian (2010),[19] Hideki Taoka *et al.* (2010),[20] Singh *et al.* (2015),[6] Ranjana and Sadhna (2016),[4] and Sharma *et al.* (2016).[14]

In the present study, in CPS out of 30 NILM cases, three cases were of trichomonas, two cases of bacterial vaginosis and candida each, while in LBC out of 36 NILM cases, six cases of trichomonas, five cases of candida and three cases of bacterial vaginosis. In our study, detection of infectious organism was higher (39%) in LBC as compared to 23.4% in CPS.

Figure 6 shows comparison of Photomicrograph finding of CPS & LBC pap smear, of a case of trichomonas vaginalis showing rounded or ovoid cyanophilic organism, the organism may be better visualized on liquid based preparations

This finding is supported by study done by Afsan *et al.*[8] This finding is in discordance with the study of Sharma *et al.* (2016).[14]

Table 8 shows that in present study sensitivity of LBC (78.6%), for detection of a histological proven lesions (CIN I/ LSIL) was significantly higher then CPS (71.4%) . Similar observation was reported by Adhemar Longatto Filho *et al.*[21] and Beerman *et al.*[2], they observed that Liquid based cytology had a significantly higher sensitivity than the conventional Pap to detect LSIL+ lesions. Arbyn *et al.*[22] reported similar sensitivity and specificity of two methods. This is in discordance with our study. Our study also showed significantly higher specificity of LBC (100%) then CPS (95.8%) when using LSIL as a cytological cutoffs. This is in discordance with Arbyn *et al.*[22] and Beerman *et al.*[2], They reported similar specificity between the two methods. Adhemar Longatto Filho *et al.*[21], reported that specificity of conventional smear in their study (88.2%) was significantly higher then LBC (75.4%) considering LSIL+ lesions . This finding is in discordance with our study.

Table 9 shows that in present study, sensitivity of LBC (72.7%) was significantly higher than CPS (63.6%) 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 Bergeron *et al.*[5], Adhemar Longatto Filho *et al.*[21], Afsan *et al.*[8], Jie Zhu *et al.*[24] and Beerman *et al.*[2] found that in their studies sensitivity of LBC were higher then CPS, this is consistent with our study. Adhemar Longatto Filho *et al.* [21] observed that Liquid based cytology had a significantly higher sensitivity (91.3%) than the conventional Pap (72.8%) to detect HSIL+ at histology. Study done by Taylor *et al.*[23], of 5652 cases, shows that CPS & LBC performance and accuracy were statistically similar in their study. This finding is in discordance with our study. Large meta-analyses by Arbyn *et al.* [22] 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+, these findings are in discordance with our study.

CONCLUSION

LBC shows an almost complete elimination of most causes for unsatisfactory CPS such as mucin, hemorrhage, inflammatory cells with scant cellularity remaining as the

sole cause for unsatisfactory LBC. Using LBC, it was possible to detect infective organism even when their load was low. 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 for detection of LSIL and HSIL lesions. Considering the higher cost of LBC, especially in low-resource setting like ours, it could not be generalized but wherever it is feasible LBC can be used instead of conventional cytology.

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