

CLINICAL ASSESSMENT AND CORRELATION OF PAP SMEAR AND LIQUID BASED CYTOLOGY IN BAD CERVIXKhushboo Verma¹**HOW TO CITE THIS ARTICLE:**

Khushboo Verma. "Clinical Assessment and Correlation of PAP Smear and Liquid Based Cytology in BAD Cervix". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 53, October 16; Page: 12277-12287, DOI: 10.14260/jemds/2014/3623

ABSTRACT: AIM: Aim of our study to analyze the prevalence of premalignant lesion based on Pap smear and liquid based cytology in patients of bad cervix. **OBJECTIVE:** To compare sensitivity and specificity of two methods for screening of bad cervix and to know IDR (increase detection rate) of cervical cancer by conventional Pap smear and liquid based cytology. **MATERIAL AND METHOD:** 200 women attending Gynaecology OPD were random selection on the basis of inclusion criteria. All 200 selected women were subjected for down staging through per speculum examination for identification of bad cervix. Pap smears of all 200 selected women were taken and ensured that no local douche, antiseptic cream and no local internal examination was done on the day of test. The prepared smears were then stained according to Papanicolaou's technique. Liquid based cytology smears preparing by using cervical brush 1-1.5cm were inserted into the cervical os until the large outer bristles of the brush touch ectocervix. Data collected for sociodemographic, parity, down staging clinical examination Pap smear and LBC was organised, interpreted and analysis on appropriate statistical software. P value < 0.05 is considered significant **RESULT:** Analysis revealed that the maximum number of women in our study are from middle age group(31-40yrs), low socioeconomic status, married before 18 yrs, multipara, do not use any contraceptive, uneducated, urban, Muslim population.in this study more abnormal smear is seen in LBC as compared to pap smear. Sensitivity and specificity for LBC is more as compare to pap smear. **CONCLUSION:** In low resource setting like ours were facilities for radiology, chemo- radiotherapy and supportive care are limited or unavailable. It is important to identify which resources fill healthcare need most effectively and to consider alternative approaches, LBC is strongly advocated in the best interest of public health, by improving the quality of the sample and reducing the likelihood of false negative cytology result. Thus it will significantly improve early detection and treatment of cervical lesions and reducing morbidity and mortality due to cancer cervix.

KEYWORDS: Cervical cancer, liquid based cytology, compare pap smear and liquid based cytology.

INTRODUCTION: Cancer of the uterine cervix is a global problem. Cervical cancer is the second most common cancer in women world-wide. With an estimated 500, 000 new case occurring annually and 275, 000 deaths occurring each year.¹ And it is the most common cancer in women in the developing countries, globally, 15% of all cancer in females are cervical cancer while in Southeast Asia, cancer cervix accounts for 20%-30% of all cancers. Cancer of cervix is a major cause of death in women living in developing countries.² The good news is that 92% of cases can be detected and treated if women undergo regular pelvic examination, Pap test and liquid based cytology.³

International Agency of Research on Cancer (IARC) reported 93%reduction in cervical cancer incidence when women aged 35 to 64 years were screened for 1 to 3 yearly, 84% reduction when screened 5 yearly and 64% reduction when screened 10 yearly⁴.

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India 132, 000 new cases are reported annually with 74, 000 deaths occurring each year hence, every 7 minutes a women die due to cervical cancer.³

Cancer cervix is strongly linked with an early onset of sexual activity and multiple sexual partners. Cervical cancer has significant morbidity and mortality if it is not detected before it reaches an advanced stage with symptoms. If the disease is detected in an early stage it is nearly always curable by surgery or radiotherapy. According to the program for Appropriate Technology in Health (PATH) an international non-government organization, "An important reason for the higher incidence in developing countries is the lack of effective screening programs to detect precancerous condition and treat them before they progress to cancer"

The writings of Hippocrates (approximately 450 B.C) include a description of disease of women. It was this work that first mentioned cancer of the uterus, describing a gloomy prognosis. The Hindus (5th century B.C) also included in thesis text a chapter devoted to disease of genital organ, describing tumour of vagina and cervix.⁵

Astrucet al, 1762 in 'A' treatise on the disease of women' described uterine cancer and recommended method of treatment. In 1793 Baillic in his text book of morbid anatomy included descriptions of diseases of the uterus and cervix and gave a detailed account of cancer of cervix^{6,7}

Well established screening programs for early detection of carcinoma cervix are present in developed countries which are contrary to the scenario in developing countries like India where there is lack of infrastructure, trained health personal and financial constraints and ignorance of health leads to detection of cancer cervix in advance stage. Keeping in the view, WHO has recommended screening of cervical cancer, namely down staging, which can be further evaluated by Pap smear and liquid based cytology.

Conventional Papanicolaou (Pap) smears are an effective means of screening for cervical malignancy in general population in developing countries. However there are unrealistic expectations about its performance which entails false negative results even in optimal Screening programs.

Liquid based cytology (LBC) has recently come up as an improvement over in the detection of intraepithelial lesion. Several reports have discussed benefits of LBC to conventional method of Pap smear. In most of these reports, a significant rise in sensitivity was achieved with Liquid based procedure without major losses in specificity. Some studies have shown more accurate diagnosis of endocervical lesion too.

The single most important advantage is the opportunity to use the residual sample for additional testing, including molecular studies for the presence of infection agents HPV-DNA. There are numerous advantages of LBC over conventional Pap smear. LBC reduces the proportion of inadequate smears, caused by obscuring factor like blood and inflammation.

It leads to an increase in the number of cellular abnormalities detected because it forms a mono layered suspension of cell with minimal overlap. LBC reduces the screening time, resulting greater efficiency of the technical observer who can handle a larger number of preparations.

MATERIAL AND METHODS: After obtaining informed consent and approval of the institutional ethics committee, this cross sectional study was carried out in Department of obstetrics and Gynaecology, Sultania Zanana Hospital, Gandhi Medical College, and Bhopal from 1 August 2012 to 31 July 2013.

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200 women attending Gynaecology OPD were random selection on the basis of inclusion criteria.

INCLUSION CRITERIA-1: Women who are above 18 years of age, 2 sexually active, 3 Had complaints (like white discharge per vaginum, bleeding per vaginum, inter menstrual bleeding, pain in lower abdomen, post coital bleeding)

EXCLUSION CRITERIA-1: Unmarried, 2 Prior hysterectomy or procedure on cervix (like pap smear cryotherapy and cervical biopsy)

All 200 selected women were subjected for down staging through per speculum examination for identification of bad cervix. A detailed history and thorough clinical examination was done. The woman was placed in dorsal lithotomy position. After proper positioning of the woman, gently introduced a sterile vaginal speculum and opened the blades of the speculum to view the cervix. Identify the external os by look for bleeding on touch, bleeding erosion, hypertrophied oedematous cervix, congested stippled cervix and growth or ulcer over cervix

Pap smears of all 200 selected women were taken and ensured that no local douche, antiseptic cream and no local internal examination was done on the day of test. The longer projection of the Ayre's spatula was placed in the cervix near squamo-columnar junction and rotated through 360°. The cellular material thus obtained was quickly, but gently smeared on a clean glass slide. The glass slide was then immediately put into the coplin jar containing 95% ethyl alcohol which acted as a fixative. The prepared smears were then stained according to Papanicolaou's technique.

Liquid based cytology smears preparing by using cervical brush 1-1.5cm were inserted into the cervical os until the large outer bristles of the brush touch ectocervix. It was rotated three full turn in a counter clock wise direction. End of the brush was snapped off leaving end of brush inside tube. The cap was replaced on tube.

All Collected sample was centrifuged at 2000rpm for 5 minutes in cytospin, slide were further fixed by dipping in 95% alcohol for minimum 30 minutes, and finally the slide were stained with the pap stain. The cytological interpretation of the smears was made according to the New 2001 Bethesda system.

Data collected for socio demographic, parity, down staging clinical examination Pap smear and LBC was organized, interpreted and analysis on appropriate statistical software. P value < 0.05 is considered significant.

RESULT AND ANALYSIS: In this study maximum no. of women where in age group of (31-40) middle age group followed by (20-30), (41-50) and (50-60) that is 35%, 25%, 17% and 13%, Mean age of women are 38.2 +- 10, mean age with standard deviation (20-60). [TABLE-1].

Maximum no. of cases was of low socioeconomic status according to modified Prasad's classification. [TABLE-2].

In present study maximum no. of cases were married before 18 year [TABLE-3].

In present study maximum no. screened were multipara. [TABLE-4].

Maximum no of abnormal cytology seen in patient who had not use any contraceptive method 9 cases with LBC and 6 cases with Pap smear.[TABLE-5].

Maximum no of cases in present study was uneducated 127 and educated 73 cases in which 13 and 4 abnormal cell with LBC, 1 and 4 each with Pap smear. [TABLE-6].

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Maximum no of cases in present study is urban 113 cases and rural 87 cases in which 6 and 11 abnormal cytology with LBC, 2 and 10 abnormal cytology with Pap smear. [TABLE-7].

Maximum no of cases in present study is Muslim 108 cases and Hindu 92 cases in which 10 and 7 abnormal cell with LBC, 6 each with Pap smear [TABLE-8].

In this study 12 Abnormal Smears with epithelial cell abnormality includes ASC US, ASC H, LISL, HSIL and SCC pap smear and 17 in LBC. [TABLE-9].

Comparison of diagnosis on LBC V/S Pap smear shown in TABLE-10.

In present study of LBC cervical smear the most common lesion in NILM category was inflammatory smear+- reactive changes (50.83% cases), squamous metaplasia (2.72%), bacterial vaginosis (4.9%), trichomonas vaginalis (1.1%) [TABLE-11].

In present study of pap smear the most common lesion in NILM category was inflammatory smear+- reactive changes (58.01% cases), squamous metaplasia 4.42%), bacterial vaginosis (3.31%), trichomonas vaginalis (16%)[TABLE-12].

The most common abnormal lesion was ASC-H (52.94%) and Squamous cell carcinoma accounting for (17% and 5.88%) each, LSIL and HSIL was 11.7% [TABLE-13]

The most common abnormal lesion was ASC US (25%) and ASC-H (25%) and Squamous cell carcinoma accounting for (25% and 8.33%) each, LSIL 16.67% [TABLE-14]

DISCUSSION: The present study was carried out on 200 patients selected by down staging attending the gynaecology OPD Insultaniazanana hospital, Gandhi medical college, Bhopal. In all these patients cervical cytology was done by both Pap smear and liquid based cytology.

In present study 70 cases (35%) were in 31-40age group. Minimum age of patient screened was 20yrs and maximum was 60 yrs. Mean age of women were 38.2 ± 10. SD between 20-60 yrs. This is comparable to study by Betashet.al (2008)⁸ in which mean age was 39 years and in contrast to study by Rmiene⁹ et al (2010) in which mean age was 42.4 years

In present study majority of cases i.e., 99 cases (49%) belong to the class III & V (modified Prasad classification) out of which there were 17 abnormal cases by LBC, of which 10 were of low socioeconomic status. Pap smear showed 12 abnormal cases out of which 7 were of low socioeconomic status. This finding is similar to that noted by Christopherson WM and Parker JE et al (1960),¹⁰ who showed a higher incidence of disease in women of low socio-economic class.

In present study 101 cases (50.5%) were married before 18 yrs, in which 12 cases had abnormal cytology by LBC and 9 by Pap smear. On other hand 5 and 3 cases had abnormal cytology with LBC and Pap smear, who were married after 18yrs. This is comparable to study by Kerkar RA, Shanta V et al,¹¹ who found that early initiation of sexual relationship was associated with increased risk of developing cancer cervix.

In present study majority of the cases with abnormal cytology had multiparity, 16 and 10 abnormal cytology seen in LBC and pap smear; a finding concordant to the studies of Shankar Narayana et al(2003)¹² and Parker et al(2001),¹³ who found that grand multi parity had a fourfold increased risk of developing cervical cancer

The most common presenting complaint in present study was white discharge per vaginum in 136 cases (68%). Kenneth and Yao et al (2002)¹⁴ also had emphasized on the significance of vaginal discharge and its association with neoplastic changes in the cervix.

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LBC also plays a major role in the increased detection of infectious agents over conventional Pap smear. This is due to removal of obscuring factors like blood, mucus and inflammatory cells. In present study, we found 16 lesions of infectious diseases by LBC comprising of 9 cases of bacterial vaginosis, 5 squamous metaplasia and 2 cases of trichomonas vaginalis. Pap smear detected only 6 cases of bacterial vaginosis, 3 cases of trichomonas vaginalis and 1 case of candida albicans. Which is comparable to study by Papilto JI et al (1998)¹⁵ and lee et al (1997).¹⁶

Maksem JA et al (2001)¹⁷ and Baker J (2002)¹⁸ who has shown increased detection rate of LSIL (1.8%), HSIL (4.4%) by LBC as compared to LSIL (0.7%) and HSIL (1.3%) by CPS. Also Baker J et al (2002) showed (5.1%) LSIL and (1%) HSIL detection by LBC as compared to (3.5%) LSIL and (0.7%) HSIL detection by CPS. These results are comparable with present study showing LSIL (1%) and HSIL (1%) by LBC compared to LSIL (.5%) and HSIL 00% by CPS

In present study there were 9 case (4.5%) of ASCUS in LBC while 3 cases (1.5%) in pap smear, this is comparable with Maccallini V et al (2008)¹⁹ who found that atypical squamous cells of undetermined significance and atypical glands of undetermined significance reports were more frequent in CPS vs. LBC. Also Maksem JA et al (2001)¹⁷ showed ASCUS were 3.3% in LBC vs. 1.8% in CPS. Better preservation of abnormal cells on LBC slide allows for more definitive categorization of abnormal cells as intraepithelial lesions rather than classifying them in equivocal ASCUS category.

Authors	Sensitivity		Specificity	
	LBC	Pap smear	LBC	Pap smear
Present study	80	52	99	98
Lee et al (2006) ²⁰	71.4	84.1	57.1	89.7
Sherwani et al (2007) ¹⁵	97.6	53.7	50	50
Betash N et al (2008) ⁸	83	66	98	86
Deshou H et al (2009) ²¹	95.4	78.9	-	-
Rimierie et al (2010) ⁹	78.1	68.7	91.8	93.8

There are 2 possible explanations for improved sensitivity with LBC:

1. LBC improves sample collection by markedly increasing number of cells that leave collection device into vial.
2. LBC reduces obscuring elements like blood and mucus thus allows better detection of abnormal cells that could have been otherwise hidden on CPS.

The advantages of liquid-based cytology are mainly that by the liquid based method, the specimen is collected in a preservative solution and allows for long term storage of the liquid sample. By giving a clear background and removal of contaminating mucus and blood, liquid based cytology improves the quality of screening of slides and also it reduces reading time. The sample enrichment which can be possible in a liquid preservation media helps in harvesting a more representative sample.

CONCLUSION: In low resource setting like ours were facilities for radiology, chemo- radiotherapy and supportive care are limited or unavailable. It is important to identify which resources fill healthcare need most effectively and to consider alternative approaches, LBC followed Pap smear

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model if adopted can be answer to this (krishnakumar duraisamy et al). Hence, sequential testing has a better diagnostic accuracy than parallel testing.

LBC is strongly advocated in the best interest of public health, by improving the quality of the sample and reducing the likelihood of false negative cytology result. Thus it will significantly improve early detection and treatment of cervical lesions and reducing morbidity and mortality due to cancer cervix.

Age wise distribution of cases		
Age Group	Number of Cases	Percentage (%)
20-30	50	25
31-40	70	35
41-50	53	27
50-60	27	13
Total	200	100

Table 1

Maximum no. of women where in age group of (31-40) middle age group followed by (20-30), (41-50) and (50-60) that is 35%, 25%, 17% and 13%, Mean age of women are 38.2 +- 10, mean age with standard deviation (20-60).

Socio-Economic Status						
Sr. No	Category	No. of Cases	CP		LBC	
			Normal	Abnormal	Normal	Abnormal
I	more then 1500	7	7	0	7	0
II	1000-1499	44	42	2	41	3
III	500-999	55	52	3	51	4
IV	200-499	30	27	3	26	4
V	less than 200	64	60	4	58	6

Table 2

In present study maximum no. of cases were of low socioeconomic status according to modified Prasad's classification.

Age at Marriage					
Category	No. of Cases	CP		LBC	
		Normal	Abnormal	Normal	Abnormal
Less than 18	101	92	9	89	12
More than 18	99	96	3	94	5

Table 3

In present study maximum no. of cases were married before 18 year

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parity	No. of Case	CP		LBC	
		Abnormal	Normal	Abnormal	Normal
Nulligravida	5	0	5	0	5
1 to 2	49	2	47	1	48
3 to 4	105	4	101	7	98
5 or more	41	6	35	9	32

Table 4

In present study maximum no. screened were multipara.

Association of cervical lesions with risk factor				
Contraception Used	Pap smear		LBC	
	NILM	abnormal	NILM	Abnormal
OCP	51	1	49	3
Condom	42	0	42	0
IUCD	15	0	14	0
Tubectomy	57	5	58	4
No Contraception used	23	6	20	9

Table 5

Maximum no of abnormal cytology seen in patient who had not used any contraceptive method 9 cases with LBC and 6 cases with Pap smear.

Type	No. of Case	Pap smear		LBC	
		Abnormal	Normal	Abnormal	Normal
Uneducated	127	11	116	13	14
Educated	73	1	72	4	69

Table 6: Association of cervical lesions with Education

Maximum no of cases in present study was uneducated 127 and educated 73 cases in which 13 and 4 abnormal cell with LBC, 1 and 4 each with Pap smear.

Area	No. of Case	Pap smear		LBC	
		Abnormal	Normal	Abnormal	Normal
Rural	87	10	77	11	76
Urban	113	2	111	6	107

Table 7: Association of cervical lesions with Residence

Maximum no of cases in present study is urban 113 cases and rural 87 cases in which 6 and 11 abnormal cytology with LBC, 2 and 10 abnormal cytology with Pap smear.

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Type	No. of Case	Pap smear		LBC	
		Abnormal	Normal	Abnormal	Normal
Hindu	92	6	86	7	85
Muslim	108	6	102	10	98

Table 8: Association of cervical lesions with religion

Maximum no of cases in present study is Muslin 108 cases and Hindu 92 cases in which 10 and 7 abnormal cell with LBC, 6 each with Pap smear.

Distribution of cases into type of lesion		
Lesions	Pap smear	LBC
NILM*	179	181
Abnormal*	12	17
Unsatisfactory smears***	9	2
Total	200	200

Table 9

NILM= negative for intraepithelial malignancy

Abnormal = Smears with epithelial cell abnormality includes ASC US, ASC H, LISL, HSIL and SCC.

Unsatisfactory for evaluation because of obscuring blood, excessive overlapping of cell etc.

Comparison of diagnosis on LBC V/S Pap smear			
Sl. No.	Categories	LBC Diagnosis	PS Diagnosis
1	Negative for inflammatory or neoplastic lesions	63	55
2	Menopausal' atrophic	10	3
3	Squamous metaplasia	5	8
4	Inflammatory ± Reactive	92	103
5	Bacterial Vaginosis	9	6
6	Trichomonas Vaginalis	2	3
7	Candida	0	1
8	ASC US	0	3
9	ASC H	9	3
10	LSIL	2	2
11	HSIL	2	0
12	Squamous Cell carcinoma	3	3
13	Small cell variant of SCC	1	1
14	Unsatisfactory	2	9
	Total	200	200

Table 10

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Interpretation of Liquid based cytology- NILM category			
Sl. No.	LBC Diagnosis	No. of Lesion	Percentage
1	Negative for inflammatory or neoplastic pathology	63	34.81
2	Menopausal/atrophic	10	5.52
3	Squamous metaplasia	5	2.76
4	Inflammatory ±Reactive	92	50.83
5	Bacteria Vaginosis	9	4.97
6	Trichomonas Vaginalis	2	1.10
	Total	181	100

Table 11

In present study of LBC cervical smear the most common lesion in NILM category was inflammatory smear+/- reactive changes (50.83% cases), squamous metaplasia (2.72%), bacterial vaginosis (4.9%), trichomonas vaginalis (1.1%)

Interpretation Pap smear -NILM category			
Sl. No.	CPS Diagnosis	No. of cases	Percentage (%)
1	Negative for inflammatory or neoplastic pathology	55	30.39
2	Menopausal/atrophic	3	1.66
3	Squamous metaplasia	8	4.42
4	Inflammatory ± Reactive	105	58.01
5	Bacterial Vaginosis	6	3.31
6	Trichomonas Vaginalis	3	1.66
7	Candida	1	0.55
	Total	181	100

Table 12

In present study of pap smear the most common lesion in NILM category was inflammatory smear+/- reactive changes (58.01% cases), squamous metaplasia (4.42%), bacterial vaginosis (3.31%), trichomonas vaginalis (1.6%).

Interpretation of Liquid based cytology -Abnormal category			
Sl. No.	LBC Diagnosis	No. of Lesion	Percentage
1	ASC H	9	52.94
2	LSIL	2	11.76
3	HSIL	2	11.76
4	Squamous cell carcinoma	3	17.65
5	Small cell variant of SCC	1	5.88
	Total	17	100

Table 13

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The most common abnormal lesion was ASC-H (52.94%) and Squamous cell carcinoma accounting for (17% and 5.88%) each, LSIL and HSIL was 11.7%

Morphological distribution of abnormal lesions diagnosed on pap smear			
Sl. No.	CPS Diagnosis	No. of cases	Percentage (%)
1	ASC US	3	25
2	ASC H	3	25
3	LSIL	2	16.67
4	Squamous cell carcinoma	3	25
5	Small cell variant of SCC	1	8.33
	Total	12	100

Table 14

The most common abnormal lesion was ASC US (25%) and ASC-H (25%) and Squamous cell carcinoma accounting for (25% and 8.33%) each, LSIL 16.67%.

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