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Comparative Analysis of Visual Inspection with Acetic Acid And Lugol's Iodine And Liquiprep ™ in Cervical Cancer Screening with Cervical Biopsy As Gold Standard

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Abstract
Aim: *A modified liquid-based cytological technique referred to as the "LiquiPrep™ (LP) system" requires neither expensive equipment nor complicated specimen preparation. The aim of this study was to assess the efficacy of LP in cervical cancer screening by comparing it with VIA(Visual Inspection with Acetic acid) and VILI (Visual Inspection with Lugol's Iodine) using cervical biopsy findings as gold standard.*
Methods: *Cervical cytology specimens were collected from 200 women. LP sample was taken with the help of a Rover Cervex brush, the tip of which was broken and dropped into the alcohol based LP preservative fluid which was further processed to prepare the slide. They were later screened by VIA and VILI through colposcopy. Cytologic interpretations were classified into following categories: 1)Negative for SIL 2) Cervicitis (3) Atypical cells of undetermined significance 4) Atypical cells cannot exclude HSIL 5) LSIL 6) HSIL 7) Invasive carcinoma. VIA/VILI findings were recorded as positive or negative.*
Results: *The sensitivity was similar for both VIA/VILI and LiquiPrep™ (94.55%). However the specificity was higher for LiquiPrep™ (86.36%). LiquiPrep™ (94.55%) had a higher positive predictive value and negative predictive value (86.36%) than VIA/VILI. The percentage of false positives was higher with VIA/VILI (36.36%) compared with (13.63%). The percentage of false negatives was similar for both LiquiPrep™ and VIA/VILI (5.45%).*
Conclusions: *In conclusion, VIA/VILI can be used as an initial screening test for cervical cancer in a low resource country like India. LiquiPrep™ has a high sensitivity equal to that of VIA/VILI and a comparatively higher specificity and therefore can be used for screening in conditions when there are no financial constrains.*
Keywords: *Cervical cancer, liquid-based cytology, LiquiPrepTM, Pap smear test.*

I. Introduction

Carcinoma cervix is the fourth most common cancer worldwide with an estimated incidence of 5,28,000 cases and 2,66,000 deaths in 2012 [1] . It is one of the leading causes of death among women in developing countries and current estimates indicate that a total of 1,23,000 cases and 67,000 deaths due to cervical cancer occurred in India, contributing 23.2% and 25.2% to the global cervical cancer incidence and mortality respectively [2] . Invasive squamous cell carcinoma is preceded by precancerous changes in the cervical epithelium which are described previously as dysplasia and now as cervical intraepithelial neoplasia (CIN) . It has been firmly established that the Human papilloma virus infection plays an important role in cervical carcinogenesis [3] . Human papilloma virus are small, circular double stranded DNA viruses that belong to the papillomaviridae family. Experimental studies have identified nearly 200 types of human papilloma viruses, of those more than 40 have been identified in the genital tract and is classified into low risk and high risk categories based on the association with invasive cervical carcinoma [4]. HPV16, 18, 31, 33 and 45 are examples of high-risk types, while HPV6 and 11 belong to the low-risk types [5]. The 5 years survival rate is 90% for cervical cancer in the early stage whereas it is much lower (14%) for persons with advanced stage IV disease. The incidence of invasive cervical cancer has come down to a great extent over a span of 40 years, mainly because of early cancer detection programs. A close quarters observation on the social behaviour of our society reveals that most of the women in our country have their marriages at very early part of their life leading to early commencement of sexual activity and poor sexual hygiene which are considered to be important etiological factors for cervical carcinoma [6].

This estimate is high owing to the addition of new cases each year and also due to the fact that the diagnosed cases do not receive adequate treatment. Current resources about the natural history of cancer cervix suggest that there are two to five times women with potential precursors to cervical cancer such as those with

invasive cervical carcinoma. This results in a rough estimate of 7,000,000 women around the world with high-grade dysplasia requiring detection and treatment.

Causes of screening failure in developing countries could be attributed to the fact that a number of women with cervical cancer do not turn up for investigations and hence are excluded from the cancer registry data resulting in considerably lower estimates of statistical parameters like cancer incidence, prevalence, and disease related mortality. Morever diagnostic facilities do not reach older women or those with financial constraint which pose a great challenge in estimating the current statistics and recording the number of women with cervical cancer is problematic [6] because of the lack of organized health information systems in developing countries like India.

II. Materials And Methods

This comparative analysis was a prospective study which was conducted at Institute of Social Obstetrics and Govt. Kasturba Gandhi hospital, Chennai, attached to Madras Medical College for a period of two years. This study involved women [n=200] attending the gynaecology outpatient department, who were screened for cancer cervix using liquid based cytology followed by colposcopic screening by VIA/VILI. and cervical biopsy done if either the colposcopy findings or LiquiPrep^{TM} reports were suspicious. Ethical clearance for the study was obtained from the Institutional Ethics Committee of Madras Medical College, Chennai.

Inclusion criteria:

1. Women attending the colposcopy out patient department with symptoms of white discharge per vagina, abnormal uterine bleeding, postcoital bleeding, pruritis vulva and those with family history of gynaecological malignancy.
2. Women who are sexually active or on oral contraceptives.
3. Non pregnant women.
4. Both nullipara and multipara.

Exclusion criteria:

1. Pregnant women.
2. Menstruating women.
3. Women who had undergone hysterectomy.
4. Sexual intercourse with spermicidal jelly, douches/tampons 24 hours prior to pap smear examination.

Liquid based cytology- LiquiPrep^{TM}: The smears are timed so that they are not collected during the menstrual periods and the patient should not have intravaginal medications / douches 48 hrs before the test. The precautions to be followed include avoiding lubricants of all types and doing vaginal examination only after taking smear.

Steps in preparation of LiquiPrep^{TM} slides:

Collection of sample: The sample was taken with the help of a Rover Cervex brush, the tip of which was broken and dropped into the alcohol based LP preservative fluid.

***Concentration of the sample*:** The vial along with the tip of the brush was shaken forcefully with the help of a vortex for about 10 seconds. The contents of the vial were emptied into a 15 ml centrifuge tube. Samples that contained mucus or blood were cleared with 4 ml of cleaning solution. Centrifugation was done for approximately1000g for 10 minutes.

Preparation of the slides: The supernatant present following centrifugation was poured off. Cell base reagent was added to the sample in an amount proportional to the cell pellet formed, in accordance to the instructions by the manufacturer. A vortex was used for 10 seconds to resuspend the cell pellet. Then about 50 microlitres of the suspended cell pellet was pipetted onto a clean slide in the form of a circle of 1.5 cm diameter following which the slides are air-dried and stained by routine Pap stain.

Pap smear reporting in the hospital

1. Negative for SIL
2. Cervicitis
3. Atypical cells of undetermined significance
4. Atypical cells cannot exclude HSIL
5. Low grade SIL
6. High grade SIL

7. Invasive carcinoma

Visual inspection with acetic acid (VIA) & visual inspection with Lugol's iodine (VILI): The procedure is carefully explained to the patients, they are made comfortable and privacy ensured. The patient is placed in the lithotomy position. Good visualization ensured. Any abnormal findings in the external genitalia are recorded. Cusco's speculum is inserted into the vagina so that the cervix is clearly visualised. The discharge or mucus is wiped by means of a cotton swab wet with normal saline. The external appearance of cervix is recorded. Cervix washed using freshly prepared 5% acetic acid using a syringe. (Alternatively can be applied with cotton swab). The cervix observed for acetowhite areas after waiting for a minute. Lugol's Iodine applied by means of a cotton swab or syringe. The cervix inspected for iodine uptake areas & non uptake areas. Findings were recorded.

Charaterstics of VIA/ VILI–positive cases

(a Low grade lesion

Detection of any acetowhite areas – VIA

Detection of any non iodine uptake areas – VILI.

(B) High grade lesion

Presence of opaque acetowhite patches which appear well circumscribed, abutting the squamocolumnar junction.

Detection of thick, dense, saffron yellow or mustard yellow iodine non-uptake lesions in the tranformation zone around the

squamocolumnar juction.

Cervical biopsy

For 77 cases- 65 cases which showed abnormal results on either VIA/VILI or liquid based cytology and 12 normal cases (control), either punch biopsy or LLETZ biopsy was taken and sent for histopathological report. The biopsy reporting in our hospital is as follows.

1. No major lesion detected
2. Cervicitis
3. Mild Dysplasia-CIN 1
4. Moderate dysplasia-CIN 2
5. Severe dysplasia-CIN 3
6. Carcinoma in situ
7. Invasive Carcinoma

III. Results

The study was conducted at Institute of Social Obstetrics and Govt. Kasturba Gandhi Hospital for Women & Children, Chennai, which is a tertiary referral hospital, attached to Madras Medical College for a period of two years. 200 Patients were included in the study group and the outcome analysed using various parameters. The results were subjected to statistical analysis.

- Sample size – 200.
- Visual Inspection with Acetic Acid (VIA), done in all 200 patients.
- Visual Inspection with Lugol's Iodine (VILI) done in all 200 patients .
- Liquid based cytology was done in all 200 cases.
- Those cases showing VIA/VILI Positive (or) cytology positive were subjected to cervical biopsy.
- For 12 cases which were negative on Pap smear and also on VIA/VILI, biopsy was done (as control)

Characteristics of the study group:

70 patients (35%) enrolled in the study belonged to the age group of 31-40 years. The percentage of positive cases both on VIA/VILI and LBC was in the age group of ≥51 years (TABLES 1 & 2).

Table 1: Agewise distribution of VIA/VILI positive cases

AGE	**21-30**	**31-40**	**41-50**	**□51**
TOTAL	39	70	60	29
VIA/VILI POSITIVE	8	27	19	14
PERCENTAGE OF VIA/VILI POSITIVE CASES	20.51%	38.57%	31.67%	48.28%

Table 2: Agewise distribution of LiquiPrep™ results

AGE	**21-30**	**31-40**	**41-50**	**□51**
TOTAL	39	70	60	29
NSIL	35	48	48	15

LSIL	2	7	5	1
HSIL	2	12	7	Nil
POSITIVE FOR MALIGNANCY	Nil	3	3	13
TOTAL SIL	4	22	15	14
PERCENTAGE OF SIL	10.26%	31.43%	25%	48.28%

Among the 200 patients, 73% of the marriages in the study group were around 15-20 years (TABLE 3).

Table 3: Distribution of age at marriage in the study group

AGE (YEARS)	**15-20**	**21-25**	**26-30**	**31**
FREQUENCY	146	46	4	4
PERCENT	73%	23%	2%	2%

The most common presenting symptom in the study group was white discharge per vaginum (63%) followed by abnormal uterine bleeding (19.5%) (Fig. 1). White discharge per vagina was also the commonest presenting symptom in patient who showed dysplasia on biopsy.

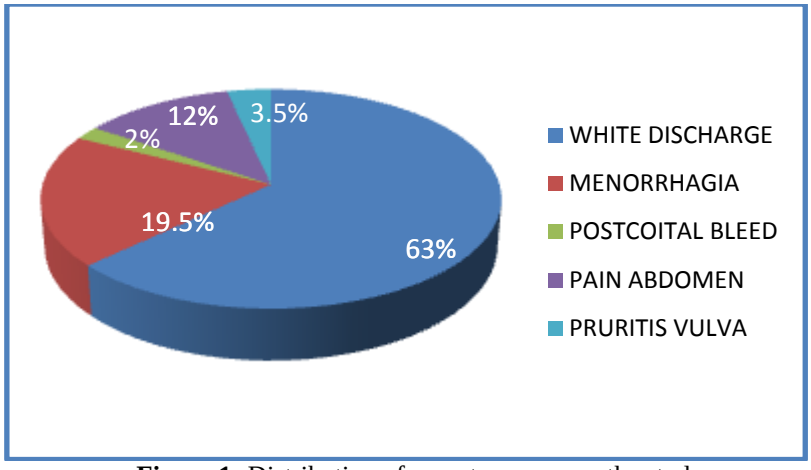

Figure 1: Distribution of symptoms among the study group

Results of VIA/VILI on colposcopy:

VIA/VILI was positive (Fig. 1 & 2) in 68 cases (34%) and 132 cases (66%) showed negative results (TABLE 4).

Table 4: Distribution of VIA/VILI positive cases in the study group

VIA/VILI POSITIVE	68 CASES	34%
VIA/VILI NEGATIVE	132 CASES	66%

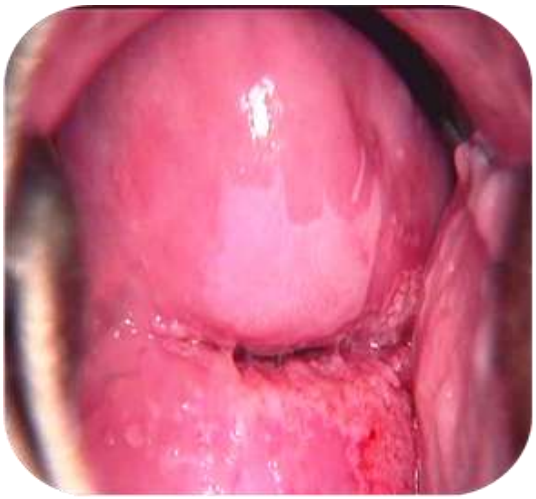

Figure 2: VIA positivity in a case of HSIL

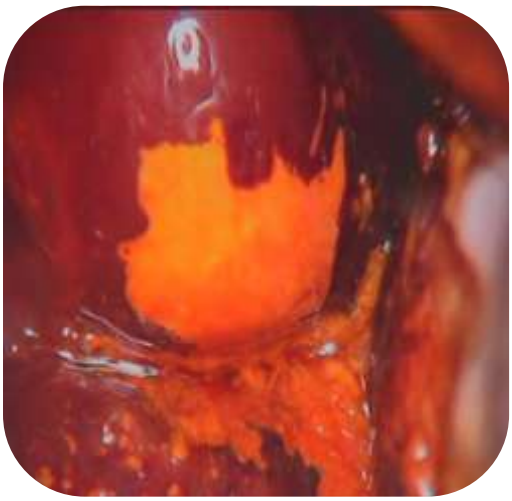

Figure 3: VILI positivity in a case of HSIL

Results of screening by Liquiprep ™:

Liquid Based Cytology report was inadequate in 3 patients (1.5%), normal in 20 patients (10%), atrophic smear in 9 patients (4.5%), Cervicitis in 113 patients (56.5%), LSIL (Fig. 4) in 15 patients (7.5%), HSIL (Fig. 5) in 21% (10.5%), SCC (Fig. 6) in 17 patients (8.5%) and adenocarcinoma in 2 patients (1%) (TABLE 5).

Table 5: Results of screening by LiquiPrep™

FINDINGS	NUMBER OF CASES	PERCENT
INADEQUATE	3	1.5%
NORMAL	20	10%
ATROPHIC	9	4.5%
CERVICITIS	113	56.5%
ASCUS	-	0%
LSIL	15	7.5%
HSIL	21	10.5%
SCC	17	8.5%
ADENOCARCINOMA	2	1%

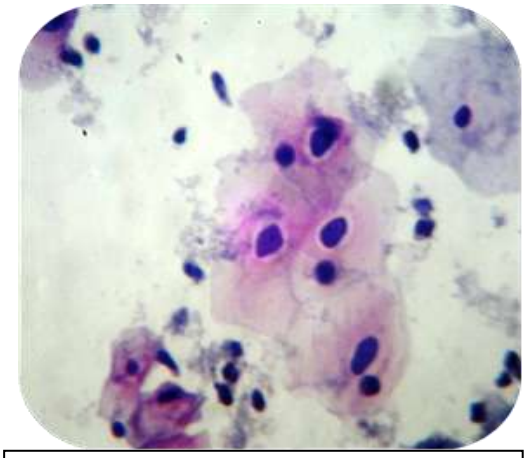

Figure 3: LiquiPrep™ smear showing Low grade squamous intraepithelial lesion

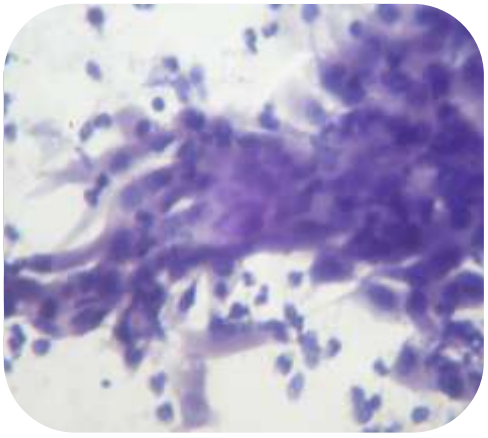

Figure 4: LiquiPrep™ smear showing high grade squamous intraepithelial lesion

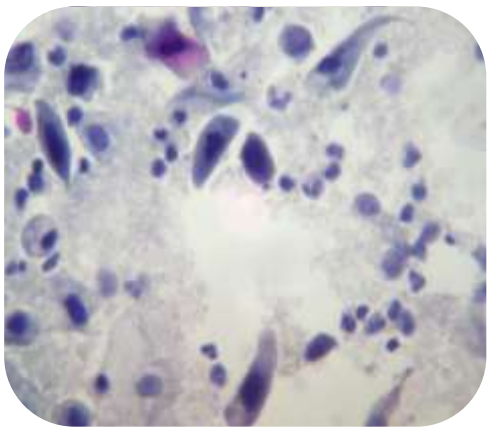

Figure 5: LiquiPrep™ smear- Invasive squamous cell carcinoma

Findings in cervical biopsy:

Biopsy done in 77 cases. 65 cases either VIA/VILI or cytology positive. In 12 cases who were VIA/VILI negative and also negative on both conventional pap and LBC, biopsy was done as control.

Biopsy was reported as cervicitis in 22 cases (28.5%), CIN 1 in 16 cases (20.8%), CIN 2 in 14 cases (18.2%), CIN 3 in 6 cases (7.8%), carcinoma in situ with focal microinvasion in 1 case (1.3%), SCC in 16 cases (20.8%) and adenocarcinoma in 2 cases (2.6%)– (TABLE 6).

Table 6: Biopsy results

FINDINGS	NUMBER OF CASES	PERCENT
CERVICITIS	22	28.5%
CIN 1	16	20.8%
CIN 2	14	18.2%
CIN3	6	7.8%
CIS	1	1.3%
SCC	16	20.8%
ADENOCARCINOMA	2	2.6%

The most common age group of CIN is 31-40 years whereas invasive carcinoma is common above the age group of 50 years (TABLE 7).

Table 7: Agewise distribution of Biopsy results

BIOPSY RESULTS	□20	21-30	31-40	41-50	□50
CERVICITIS(22)	-	1	11	6	4
CIN 1(16)	-	2	7	5	2
CIN 2(14)	-	2	7	5	-
CIN 3(6)	-	-	5	1	-
CIS(1)	-	-	-	-	1
SCC(16)	-	-	2	3	11
ADENOCARCINOMA(2)	-	-	1	-	1

IV. Discussion

Cervical cancer is one of the leading causes of morbidity and mortality among women worldwide. Many studies revealed the association of human papilloma virus infection in both precancerous and invasive cervical cancer. Most of the HPV infection are transient, if it persists the risk of developing preneoplastic lesions increases as well as the risk of developing cervical cancer [7]. So effective screening is a must to lower the incidence of carcinoma cervix in the developing countries.

Characteristics of the study group:

In this study most of the patients were in the age group 31 to 40 years (35%). In the cross sectional study done in October 1995 to August 1997women attending 15 primary health center in Zimbabwe [8] to study the efficacy of VIA and cytology in the study group, the age group included was 25 to 55 years and the mean age was 33.25. In a study evaluating the efficacy of VIA with conventional pap smear by Divya Hedge et al [9], 56.6% belonged to the 41-50 year age group and 68.5% belonged to the lower middle age group.

In this study most of the patients attained menarche at 13-14 yrs (82%) and 73% of them got married at 15-20 yrs. In the cross sectional screening test done in 15 primary health center in Zimbabwe [8], 56% attained menarche at 13 years and most of them got married at 18 years (46%).

In this study 46.5% of patients were of parity 2. In a study by SO Albert et al [10] comparing VIA and Pap smear , 79.8% patients were multiparous, while 20.2% were grandmultiparous.

The most common presenting symptom in the study group (63%) was white discharge per vagina. The common presenting symptom in women with dysplasia was also white discharge per vagina. In a study evaluating the efficacy of VIA with conventional pap smear by Divya Hedge et al [9], the major presenting complaint was menstrual irregularities in 40.8% women. 12.9% of women had complaints of white discharge per vagina. White discharge per vagina was the most common presenting complaint among patients in whom precancerous and malignant lesions were detected.

Findings on VIA/VILI:

VIA and VILI positive in 68 cases and in 132 cases the test results were negative. Thus 34% of the screening population showed positive results. The above findings correlated with the study conducted by Sankaranarayanan et al [11], in Kolkatta involving 5881 women which showed VIA positive results in 30% .

Liquid Based Cytology results:

3cases (1.5%) were inadequate, 20 cases (10%) normal, 9 smears (4.5%) were atrophic, 113 cases (56.5%) showed cervicitis, 15 cases (7.5%) were LSIL, 21 cases (10.5%) showed HSIL. 17 cases (8.5%) were squamous cell carcinomas and 2 cases (1%) were adenocarcinomas. In this study the rate of inadequate smears was 1.5%. In the study by M Tunc Canda et al [12], LiquiPrep™ smears were inadequate only in 0.1%.

Agreement between VIA/VILI and Liquid Based Cytology:

Five cases which were negative on VIA/VILI were reported as LSIL in cytology. Out of 28 low grade lesions on colposcopy, 10 were reported as LSIL and one was reported as HSIL in cytology (TABLE 8).

Table 8: Agreement between VIA/VILI and Liquid Based Cytology

VIA/VILI	LIQUID BASED CYTOLOGY			
	NSIL	LSIL	HSIL	INVASIVE CARCINOMA
NEGATIVE(132)	127	5		
LOW GRADE (28)	17	10	1	
HIGH GRADE (40)	1		20	19

Comparison of VIA/VILI results with biopsy:

Among the 20 low grade lesions, 8 were cervicitis, 10 were CIN 1 and 2 cases were diagnosed as CIN 3 on biopsy. 21 high grade lesions were reported CIN 1: 3 cases, CIN 2: 12 cases and CIN 3: 6 cases. Out of 19 cases of invasive carcinomas, 1 case was carcinoma in situ, 16 cases were reported as SCC and 2 cases were reported as adenocarcinoma on biopsy (TABLE 9).

Table 9: Comparison of VIA/VILI with biopsy

VIA/VILI	BIOPSY						
	CERVICITIS	CIN 1	CIN2	CIN 3	CIS	SCC	ADENO CARCINOMA
NEGATIVE(17)	14	3					
LOW GRADE(20)	8	10	2				
HIGH GRADE(21)		3	12	6			
INVASIVE CARCINOMA (19)					1	16	2

Efficacy of VIA/VILI as screening test:

In our study the sensitivity of VIA/VILI was 94.55%, specificity was 63.64%, Positive predictive value was 86.67% and negative predictive value was 82.35%. The sensitivity, specificity, PPV and NPV of various studies are given in TABLE 10.

Table 10: Comparison of efficacy of VIA/VILI in various studies

STUDY	SENSITIVITY %	SPECIFICITY %	PPV %	NPV %
Present study	94.55	63.64	86.67	82.35
Divya Hedge et al [9] (2011)	70.8	95	96.5	62.9
Shankaranarayanan et al [11] (2001)	90	92	17	97
Zimbabwe/JHPIEGO [8] Phase I	NA	NA	25.9	73.3
Phase II	76.7	64.1	18.6	73.3
Goel et al [13] (2005)	96.7	36.4	58	99.7
Singh KN et al [14] (2010)	93.1	86.8	22.1	99
Bhatla N et al [15] (2007)	100	53.3	15.7	100
Rana T et al [16]	93	90	62.5	98.8

Comparison of Liquid based cytology results with biopsy:

Three cases of NSIL were reported as CIN 1- 3 on biopsy. Out of 15 cases of LSIL 11 were reported as CIN 1, 3 as CIN 2 and 1 as cervicitis. 21 cases of HSIL on LBC turned out to be CIN 1- 2 cases, CIN 2-13 cases, CIN 3-6 cases. Among 19 cases of invasive carcinoma, one case was reported as carcinoma in situ with focal microinvasion, sixteen cases were squamous cell carcinoma and two were adenocarcinoma on biopsy (TABLE 11).

Table 11: Comparison of LBC results with **biopsy**

LIQUIPREP™ RESULTS	BIOPSY						
	CIN 1	CIN 2	CIN 3	CIS	SCC	ADENO CARCINOMA	CERVICITIS
NORMAL(2)							2
CERVICITIS(15)	3						12
ATROPHIC(2)							2
INADEQ(0)							
ASCUS(0)							

LSIL(15)	11	1					3
HSIL(21)	2	13	6				
INVASIVE CARCINOMA (19)				1	16	2	

Efficacy of LiquiPrep™ as screening test:

In our study the sensitivity of LiquiPrep™ was 94.55%, specificity was 86.36%, Positive predictive value was 94.55% and negative predictive value was 86.36%. The sensitivity, specificity, PPV and NPV of various studies are given in TABLE 12.

Table 12: Comparison of efficacy of Liquid Based Cytology in various studies

STUDY	**SENSITIVITY %**	**SPECIFICITY %**	**PPV %**	**NPV %**
Present study	94.55	86.36	94.55	86.36
Hussein T et al [17]	83	82	62	93
Mahmood Khaniki et al [18] (LiquiPrep™)	83	98	83	96
Longatto Filho et al [19] (ThinPrep)	33.3	100	100	88.8
Hua Chen et al [20] (ThinPrep)	80	63.16	16	97.3
Nadereh Behtash et al [21] (LiquiPrep™)	86	98.5	86	98.5
Lee HS et al [22] (ThinPrep)	79	98		
Lee KC et al [23] (ThinPrep)	85.1	98.3		
Lee KC et al [24] (Surepath TM)	91.7	75.9		
Lim YK et al [25] (MonoPrep)	94.9	92.3		

Comparison of various screening procedures:

The sensitivity was similar for both VIA/VILI and LiquiPrep™ (94.55%). However the specificity was higher for LiquiPrep™ (86.36%). Positive predictive value was more for LiquiPrep™ (94.55%). Negative predictive value was higher for LiquiPrep™ (86.36%) followed by VIA/VILI (82.35%). The percentage of false positives was highest with VIA/VILI (36.36%). The percentage of false negatives was similar for both LiquiPrep™ and VIA/VILI (5.45%) (TABLE 13).

Table 13: Comparison of efficacy of VIA/VILI and LiquiPrep™ as screening procedures

	VIA/VILI	**LIQUIPREP™**
SENSITIVITY	94.55%	94.55%
SPECIFICITY	63.64%	86.36%
PPV	86.67%	94.55%
NPV	82.35%	86.36%
PERCENTAGE OF FALSE POSITIVES	36.36%	13.63%
PERCENTAGE OF FALSE NEGATIVES	5.45%	5.45%

V. Conclusion

his correlative study of VIA/VILI and LiquiPrep™ smear with histopathological examination of cervix revealed that VIA/VILI and LiquiPrep™ had similar sensitivity of 94.55%. LiquiPrep™ had a higher specificity than that of VIA/VILI. LiquiPrep™ had higher positive predictive value and negative predictive value than VIA/VILI. VIA/VILI showed the highest percentage of false positives while the percentage of false negatives were similar for both.

In conclusion, VIA/VILI can be used as an initial screening test for cervical cancer in a low resource country like India. The false negative and false positive cases in this study can be minimized by proper screening and interpretation and further follow up by cytological smears. LiquiPrep™ has a higher sensitivity equal to that of VIA/VILI and a comparatively higher specificity compared to VIA/VILI and moreover of the ease of preparing LiquiPrep™, it can be used for screening in conditions where there are no financial constrains. Using this technique, a portion of each sample can be preserved for further studies like HPV detection and subtyping.

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