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A comparative study of liquid based cytology and visual inspection with acetic acid as screening method of premalignant lesions of cervix

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***Abstract*---**The study was conducted to evaluate the prevalence of premalignant lesions of cervix and to compare the diagnostic efficacy of liquid based Cytology and Visual Inspection with Acetic Acid in predicting pre- malignant lesions of cervix. It was a facility based cross sectional study conducted on sexually active women in the age group of 18 to 60 years. Detailed history was obtained from all the patients followed by thorough examination. Per Speculum Examination, LBC and VIA was performed in all cases using standard procedure. Women with either positive VIA or with positive Cervical

Cytology (LBC) or both positive, were subjected to cervical biopsy. A total of 2195 women were enrolled for LBC and VIA both. Liquid based cytology revealed premalignant lesions in 4.2% cases whereas VIA was positive in 7% cases. Histopathology revealed CIN 1, 2 and 3 in 22.5%, 11.1% and 4.1% cases respectively. Sensitivity of VIA was 76% and that of LBC was 55% whereas specificity of LBC was higher (70.88%) as compared to VIA (46.5%). VIA is more sensitive than the LBC for the detection of pre malignant lesions of the cervix. However, the specificity of VIA was much lower which is the major disadvantage of VIA, which leads to high rates of referral for treatment.

***Keywords*---**VIA, LBC, premalignant, cervical lesions, Sensitivity, Specificity, histopathology.

Introduction

Cervical cancer is the second most common cancer among females and is a leading cause of mortality among Indian women (Balasubramaniam et al., 2021). India accounts for more than one-fifth of the total cervical cancer deaths (Sachan et al., 2018). Cervical cancer is the fourth most common cancer in women world-wide (Arbyn et al., 2018). The primary cause for cancer cervix is HPV 16 and HPV 18 (Bhattacharjya et al., 2015). Cancer cervix remains in pre-invasive form and progresses to various grades of dysplasia before progressing to invasive cancer (Kalyankar et al., 2017). Therefore, prolonged natural history of disease provides us time to screen, treat it early and prevent the progression of disease (Joshi et al., 2015). Various screening methods for cervical carcinoma include, Conventional exfoliative cervicovaginal cytology i.e. the cervical (Pap) smear, pelvic examination, colposcopy, liquid based cytology (LBC), automated cervical screening techniques, HPV testing, visual inspection of cervix after applying Lugol's iodine (VILI) or acetic acid (VIA) (Verma et al., 2020). Screening programs for cervical cancer using the conventional Pap smear (CPS) technique have been successful in detecting cancers of the cervix significantly (Gakidou et al., 2008).

However, CPS technique has many limitations. To overcome these limitations liquid based cytology was introduced in the mid-1990, which is a better tool for processing cervical samples (Ranjana, 2016). The LBC corresponds to a sampling where cells are put in suspension in a conversation liquid (Bergeron, 2001). In LBC, suspension of cells is prepared from the sample instead of smear (as in pap smear) and the prepared suspension is then used to produce thin layer of cells on a slide (Pankaj et al., 2018, Singh et al., 2018). Sensitivity and specificity of LBC has been documented to be higher as compared to conventional cytology (Poli, 2015). The present study was conducted to evaluate the prevalence of premalignant lesions of cervix at our center and to compare the diagnostic efficacy of two methods i.e., LBC and VIA in diagnosing pre- malignant lesions of cervix.

Materials and Methods

The present study was a facility based cross sectional study conducted at the Department of Obstetrics and Gynecology in collaboration with department of Pathology, at rural tertiary care center in north India from October 2018 to March 2020. All the sexually active women in the age group of 18 to 60 years, attending the study area during the study period were included in the study after taking consent. However, Pregnant women, women with post hysterectomy status, women with current vaginal infections; known cases of Cervical cancer or with active bleeding per-vaginum were excluded from the study. Ethical clearance from the Institute's ethical committee was taken. Data of all the included patients regarding socio demographic variables were collected. Complete history including presenting complaints, menstrual cycle history, obstetrics history and past medical and surgical history was obtained from all the women and entered in data collection form. Thorough physical examination including general, systemic and local examination was done in all patients.

Per speculum and bimanual examination was also done in every case and findings were recorded. Sample for Liquid based cytology was obtained during per speculum examination with the help of a brush provided with collection bottle. After taking the sample brush rinsed in medium provided in the collection bottle for LBC. VIA was performed at the same time in those cases where any abnormal finding was seen during per speculum examination. In cases with abnormal LBC reports VIA was performed in the next visit when the patient came with an abnormal cytology report. VIA was performed by applying 5% freshly prepared acetic acid to the cervix. Acetowhite areas were noted after 60 seconds of application of acetic acid. Colposcopy guided biopsy was performed in cases of either positive VIA, abnormal LBC or both. Cervical tissue for histopathological examination was sent in cases either with acetowhite areas on VIA, with abnormal cytology report or both, after doing colposcopy. Data was compiled using Ms Excel and analysed using IBM SPSS software version 20.

Results and Discussions

The study was conducted on a total of 2195 women fulfilling the inclusion criteria over a period of 1.5 years. Majority of women (58.9% cases) in the present study belonged to the age range of 41 to 50 years and about 66.9% were residents of rural areas. Majority of women with suspected premalignant lesions were of parity 3 (41.5%) whereas only 5% women were primiparous and 1% females were nulliparous in our study (Table I). Most common presenting complaints among women in the present study was white discharge per vaginum in 88.2% cases. However, only 9.1% and 2.7% females presented with menstrual irregularities and post coital bleeding respectively. As shown in Table II per speculum examination revealed white discharge in 66.5% patients whereas inflamed cervix was observed in 10.9% cases. LBC revealed inflammatory changes in 9.4% cases, whereas ASCUS (abnormal squamous cells of unknown significance), LSIL (Low grade squamous intraepithelial lesion), HSIL (High grade squamous intraepithelial lesion), squamous cell carcinoma and adenocarcinoma were noted in 1.6%, 1.7%, 0.9%, 0.1% and 0.1% cases respectively.

VIA was positive in only 7% cases whereas it was negative in 93% cases. Histopathology was performed in 244 cases (60 patients have not given consent for HPE who had abnormality in LBC or VIA). Histopathology revealed cervicitis in the majority of females (17.2%), Cervical Intraepithelial Neoplasia (CIN) 1, 2 and 3 were documented in 11.4%, 4.9% and 3.6% cases respectively. in the patients whose LBC had finding ranging from inflammatory to HSIL. However, histopathology was suggestive of squamous cell carcinoma in 2.0% cases and adenocarcinoma in 0.8% cases in which finding of LBC was HSIL, squamous cell or adenocarcinoma (Table III). LBC correlated with histopathology in 57.8% cases of CIN I and 45.0% and 35.0% cases of CIN 2 and 3 respectively. LBC correlated with histopathology in 100% cases of squamous cell carcinoma and100% cases of adenocarcinoma. VIA was positive in 24.8%, 14.3% and 5.2 % cases of CIN 1, 2 and 3 respectively. However, VIA was positive in 100% cases of cancerous lesions of the cervix. VIA has higher sensitivity and NPV as compared to that of LBC whereas LBC was more specific and has high PPV as compared to VIA (Table IV).

Table I
Distribution of females according to demographic variables

Variables		Frequency (n=2195)	Percentage
Age group (years)	18-30	195	8.9
	31-40	198	9.0
	41-50	1292	58.9
	51-60	510	23.2
Residence	Urban	726	33.1
	Rural	1469	66.9
Parity	0	22	1.0
	1	110	5.0
	2	305	13.9
	3	911	41.5
	≥4	847	38.6

Table II
Findings of per-speculum examination and procedures in women with suspected premalignant lesion

Procedure performed	Observed results	Frequency (n=2195)	Percentage
Per-speculum findings	Normal	486	22.1
	White discharge	1459	66.5
	Inflamed cervix	240	10.9
	Suspicious cervix	9	0.4
	Cervical polyp	1	0.05
LBC	Negative	1891	86.2
	Inflammatory	207	9.4
	ASCUS	35	1.6

	LSIL	38	1.7
	HSIL	20	0.9
	Squamous cell	2	0.1
	Adenocarcinoma	2	0.1
VIA	Negative	2042	93.0
	Positive	153	7.0

Table III
Association of Histopathology with LBC and VIA

LBC	Histopathology (n=244)					
	Cervicitis	CIN 1	CIN 2	CIN 3	SCC	Adeno
Inflammatory (n=147)	0 (0)	0 (0)	2 (0.01%)	0 (0)	0 (0)	0 (0)
ASCUS (n=33)	29 (87.9)	6 (18.18%)	0 (0)	0 (0)	0 (0)	0 (0)
LSIL (n=38)	13 (34.2%)	22 (57.8%)	1 (2.6%)	2 (5.2%)	0 (0)	0 (0)
HSIL (n=20)	0 (0)	0 (0)	9 (45.0%)	7 (35.0%)	4 (20.0%)	0 (0)
Squamous cell (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100.0%)	0 (0)
Adenocarcinoma (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100.0%)
VIA						
Positive (153)	77 (50.3%)	38 (24.8%)	22(14.3%)	8 (5.2%)	6 (3.9%)	2(1.3%)

Table IV
Association of LBC and VIA with Histopathology

Test		Histopatology (244)		Total
		Positive	Negative	
LBC	Positive	55	42	97
	Negative	45	102	147
VIA	Positive	76	77	153
	Negative	24	67	91

Cancer of cervix is the most common cancer among females in India and third most common malignancy in the world. This cancer is potentially a preventable cancer which is preceded by a premalignant stage (Kalyankar et al., 2017) . Thus, it is useful to screen women for the presence of premalignant lesions. However, in a resource-limited setting where screening is opportunistic, one needs a screening test which has a high specificity (i.e. Yields high true negative) and high negative predictive value. In the present study, histopathology was conducted in 244 women. Our study documented that LBC correlated with histopathology in 40%

cases of CIN I on histopathology which were diagnosed as LSIL on LBC whereas 33.3% and 70% cases of CIN 2 and CIN 3 were diagnosed as HSIL on LBC. Out of 6 cases with squamous cell carcinoma on histopathology, LBC was suggestive of HSIL and squamous cell carcinoma in 66.6% and 33.4% cases respectively. However, LBC correlated with histopathology in 100% cases.(Table V) It was observed that in low grade lesions including ASCUS and CIN 1, the majority of patients were either misdiagnosed as cervicitis or as CIN 1 in all the studies. The reason behind this was that those cases had erosion or ulcers, hence there was inflammatory atypia for which in PAP smear those cells were considered as atypical squamous cells.

Our study revealed that VIA was positive in 69.1%, 81.5% and 80% cases of CIN 1, 2 and 3 respectively. However, VIA was positive in 100% cases of cancerous lesions of the cervix i.e. squamous cell carcinoma and adenocarcinoma. Of twenty nine VIA positive cases histopathology correlated with VIA in 16 (55.17%) cases in a study by Consul et al. (Consul et al., 2012) . Bhattacharya et. al. documented that VIA negative cases correlated well with histopathology rather than VIA positive cases (Bhattacharya et al.,2015). The findings of present study were contrasting to findings of Chaudhary et. al. where they documented higher PPV as compared to NPV (Chaudhary et al., 2014) . Overall, from the Table VI, it was observed that LBC has higher specificity and negative predictive value whereas it has lower sensitivity and positive predictive value. Our study had certain limitations such as a sample was selected from the population attending OPD which is not representative of the general population. Biopsy was taken only from those with lesions observed in VIA positive and LBC positive cases. Those with normal VIA and LBC have not undergone biopsy to confirm the negative test. These patients are assumed to be negative even in biopsy for statistical analysis.

Table V
Correlation between LBC and histology and its comparison with previous literature

LBC	Histopathology	Present study	Poudel A et. al. study (Poudel , 2015)	Alaknandaa et al. study (Alakanananda et al., 2016) .
ASCUS	Cervicitis	20.1	14	19/34
	CIN 1	10.9	10	13/34
	CIN 2	0	0	2/34
	CIN 3	0	0	0
	SCC	0	1.5	0
LSIL	Cervicitis	9	2	3/7
	CIN 1	40	11	2/7
	CIN 2	3.7	5	1/7
	CIN 3	20		0

	SCC	0	1.2	0
HSIL	Cervicitis	0	1	0
	CIN 1	0		
	CIN 2	33.3	4	6/19
	CIN 3	70		7/19
	SCC	20		3/19
Squamous cells	CIN 2	0	40	0
	CIN 3	0		0
	SCC	100	3	6(100%)
Adenocarcinoma		100	100	3(100%)

Table VI
Comparison of the Sensitivity, Specificity, Predictive values and accuracy of liquid-based cytology and VIA with respect to Biopsy in Different studies

	Study	Diagnostic accuracy	Sensitivity	Specificity	PPV	NPV
LBC	Present study	64.3	55	70.8	56.7	69.4
	Kaur et.al. (Kaur B et al., 2017) .	-	61.7	55.1	39.7	75
	Khatun et. al (Khatun S, 2019)	71	11.5	91.9	33.3	74.7
	Chaudhary et. al.(Chaudhary RD et al., 2014)	76	25	99.3	94.1	74.3
VIA	Present study	58.6	76	46.5	49.7	73.6
	Bhattacharya et. al. (Bhattacharyya AK et al., 2017)	87	89	87	32	99
	Consul et. al. (Consul S, 2012)	-	84.2	55.2	55.2	84.2

Conclusions

As a screening test, the LBC has been found to have a low sensitivity resulting in a high false-negative rate. However, its specificity is high. The sensitivity of the LBC has been found to be even lower in developing countries. Screening by LBC is not feasible in low resource settings due to several limitations like inadequate coverage of large populations, lack of infrastructure and resources required for cytological screening. VIA is more sensitive than the LBC for the detection of pre malignant lesions of the cervix. However, the specificity of VIA was much lower which is the major disadvantage of VIA, which leads to high rates of referral for treatment. VIA is easy to learn and simple, less reliant on infrastructure, low cost, point-of-care diagnosis and treatment. VIA can be combined with LBC to improve the efficacy of screening procedures in detection of pre-malignant lesions of the cervix.

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