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Original Research Article

A comparison of cervical cancer screening methods: pap smear, liquid based cytology and VIA VILI

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ABSTRACT

Background: There are multiple screening methods available for screening cervical cancer with their advantages and disadvantages, researches that compare the sensitivity, specificity, positive and negative predictive values of these tests in a low-cost setting specific to a geographical area is lacking and has to be enhanced. The objective of this study was to determine and compare the agreement, sensitivity, specificity and predictive values of conventional Pap smear cytology, liquid-based cytology and VIA/VILI with cervix biopsy.
Methods: This cross sectional study was conducted on women of >35 years of age. Pap smear specimen was collected followed by Liquid Base Cytology (LBC) using cytobrush specimen after which visual inspection with 5% acetic acid (VIA) and visual inspection with Lugol's Iodine (VILI) procedure was carried out and followed by cervix biopsy. All methods were analyzed for accuracy.
Results: Sensitivity was lowest for PAP smear (39.1%, 95% CI 19.18% to 59.1%), followed by VIA VILI (95.7%, 95% CI 87.32% to 100%), and highest for LBC (100.0%). The negative predictive value was lowest for PAP smear (87.4%, 95% CI 81.21% to 93.6%), followed by VIA VILI (99.0%, 95% CI 96.99% to 100.0%) and highest for LBC (100%). The overall diagnostic accuracy was lowest for PAP smear (88.3%) followed by VIA/VILLI (99.2%) and highest for LBC (100%).
Conclusions: Treatment decisions based on findings of the PAP smear have to be taken with caution, considering the lower sensitivity. Wherever resources are available more accurate screening methods like liquid-based cytology must be used.

Keywords: Liquid-based cytology, Pap smear, Visual inspection with 5% acetic acid, Visual inspection with Lugol's iodine

INTRODUCTION

Cervical cancer is the fourth commonest cancer affecting women worldwide and has also the seventh position of all malignancies. Globally, the less developed regions hold the majority of its burden (85%) and it accounts for about 12% of all female cancers in these countries. Cervical cancer remains the most common cancer in women of eastern and middle Africa.[1] About 87% of cervical cancer deaths occur in less developed countries. In high income countries the incidence and mortality from cervical cancer appears to be following a declining trend particularly where there are systematic screening programs. For cervical cancer to surge, it is imperative for the uterine cervix to be infected with high risk strains of human papilloma virus (HPV). The compounding of additional risk factors to HPV infection is indispensable to cause cancer.[2] The infection abides in only few sets of

women which in most cases vanishes instinctively. The women of the former group are in jeopardy as these lesions may evolve into "high-grade cervical intraepithelial neoplasia (CIN) grades 2 or 3 and adenocarcinoma in situ, which are the cancer precursors".[3]

Evermore the base of screening programs is formed targeting the women entering a sexual phase of their life and those bagging additional risk factors. The HPV test and pap test are the ones that are normally done in the Gynecology out-patient departments (OPDs). The HPV test (hybrid capture-2 or HC2) checks for a woman with an HPV infection which if positive, may mean that there are precancerous changes in the cervix. The B probe of HC2 is capable of determining 13 different HPV serotypes from the sample. HC2 uses the signal amplified method of HPV DNA identification whereas, polymerase chain reaction (PCR) uses target amplified method.[4] The Papanicolaou (Pap) test of two types, viz., conventional cytology and liquid-based cytology which checks for whether cells in the cervix are abnormal and thusly are classified by Bethesda grading system.[5]Abnormal smears will be subjected to either "repetition of the cytology, HPV triage or colposcopy".[6] Visual inspection with acetic acid (VIA) and Visual inspection with Lugol's iodine (VILI) are the other screening methods in practice to identify cervical premalignant lesions. Despite understanding well organized screening programs using Pap smears with three- to five-year screening intervals results in reduction in mortality from the disease by up to 80%, researches that compare the sensitivity, specificity, positive and negative predictive values of these screening tests in a low- cost setting specific to a geographical area is lacking and has to be enhanced.[7] The purpose of this study is to determine and compare the validity of conventional Pap smear cytology, liquid-based cytology, and VIA/VILI considering cervix biopsy as the gold standard.

The aims and objectives of this study were to determine and compare the agreement, sensitivity, specificity and predictive values of conventional Pap smear cytology, liquid-based cytology and VIA/VILI with cervix biopsy.

METHODS

A Cross-sectional study included women aged more than 35 years of age. Pregnant women, women who had underwent testing for cervical pathology in last 1 year and women who was treated for any known cervical pathology in last 1 year were excluded from the study. The data collection for the study was done between June 2016 to June 2017, for a period of 1 year. The study has included a total of 120 subjects. The sample size was calculated assuming the sensitivity of 88% for LBC as per a study by Chen C et al.[26]

To be able to detect 10% difference in sensitivity with 80% power and 5% alpha error using chi square test to detect the difference between two proportions, the total required sample size will be about 120 subjects. The sample size was calculated using static software version 13.

All the eligible women were selected into the study by purposive sampling, till the sample size is reached. After obtaining the informed written consent, all the study participants were evaluated by detailed clinical history and physical examination. Pap smear specimen was collected by an Ayers spatula and smeared on a slide and fixed with 95% of ethanol (conventional Pap smear cytology (CPAP). Liquid-based cytology (LBC) was done using Cytobrush specimen and was collected in a vial containing preservative for liquid-based preparation processing After the Pap smear and LBC is collected Visual inspection with 5% acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) procedure was carried out and following that cervix biopsy was taken. Abnormal areas in cervix turn acetowhite in VIA and yellow in VILI.

Clearance was obtained from the ethical committee, written and informed consent was sought from the patients and their attendants. They were given the option of quitting from the study if so desired by them. No element of compulsion was exerted. All data were kept confidential.

Statistical analysis

Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency, and proportion for categorical variables.

Data was also represented using appropriate diagrams like bar diagram, pie diagram, and box plots. The association between explanatory variables and HPE findings was assessed by cross-tabulation and comparison of percentages. Odds ratio along with 95% CI are presented. Chi square test was used to test statistical significance.

Univariate binary logistic regression analysis was performed to test the association between the explanatory variables and outcome variables. Unadjusted odds ratio along with 95% CI are presented. Variables with statistical significance in univariate analysis were used to compute multivariate regression analysis. Adjusted odds ratio along with their 95% CI is presented.

The utility of various screening tests in diagnosing malignancy was assessed considering HPE findings as the gold standard. The sensitivity, specificity, predictive values and diagnostic accuracy of the screening test with the decision to cut off values along with their 95% CI were presented. Reliability of the screening test was assessed by kappa statistic along with its 95% CI and p value. P value <0.05 was considered statistically

significant. IBM SPSS version 22 was used for statistical analysis.[27]

RESULTS

The following observations were made in present study and the results were analysed. The mean age was44.09 ±5.19 years in the study population, the lowest age was 35 years and the highest age was 57 years. (95% CI 43.15 to 45.03). The mean age at marriage was 18.32±2.63 years, the lowest age was 13 years and the highest age was 25 years. (95% CI 17.84 to 18.79), and mean duration of married life was 25.58 ± 6.63 years with minimum of 14 years and maximum of 45 years in the study population (95%CI 24.38 to 26.78). Among the study population the number of women in parity P1, P2, P3, P4, P5 and P6 were 3 (2.50%), 69 (57.50%), 30 (25.00%),8 (6.67%), 7 (5.83%), and 3 (2.50%) respectively. The majority 88 (73.33%) of the study population underwent tubectomy. The proportion of women using a barrier method of contraception and OCPS was 7(5.83%) and 3(2.50%) respectively. Regarding socio economic status 66 (55.00%) were in lower class, 50 (41.67%) were in middle class and 4 (3.33%) were in upper class (Table 1).

Table1: Demography.

	Mean±SD	95% CI
Age	44.09±5.19	43.15 to 45.03
Age at marriage	18.32±2.63	17.84 to 18.79
Years of married life	25.58±6.63	24.38 to 26.78
Socioeconomic status	Frequency	Percentages
Lower	66	55.00
Middle	50	41.67
Upper	4	3.33
Contraception	Frequency	Percentages
Barrier	7	5.83
OCP	3	2.50
Tubectomy	88	73.33
None	22	18.33

Among 120 subjects 67 (55.83%) were presented with white discharge PV and in 53 subjects (44.17%) there was no complaint of white discharge PV.

There was a history of post coital bleeding PV in 27 (22.50%) subjects and no history of post coital bleeding PV in 93 (77.50%) subjects. Similarly, there was a history of inter menstrual bleeding PV in 24 (20.00%) subjects and no history of inter menstrual bleeding PV in 96 (80.00%) subjects. Clinically on per speculum examination cervical erosion was seen in 24 (20.00%) subjects, white discharge PV in 36 (30.00%) and normal in 60 (50.00%) subjects (Table 2).

On PAP smear examination 4 (3.33%) had ASCUS, 3 (2.50%) had candida non-albicans, 4 (3.33%) had HSIL, 27 (22.50%) had inflammatory, 1(0.83%) had LSIL and in 81 (67.50%) subjects it was normal. Overall 9 (7.50%) had premalignant and malignant lesions and non-malignant lesions in 111 (92.50%) subjects (Figure 1).

Table 2: Clinical presentations.

White discharge PV		Frequency	Percentage
Yes		67	55.83
No		53	44.17
Post coital bleeding PV	Yes	27	22.50
	No	93	77.50
Intermenstrual bleeding PV	Yes	24	20.00
	No	96	80.00
Per speculum examination	Cervical erosion	24	20.00
	Normal	60	50.00
	White discharge PV	36	30.00

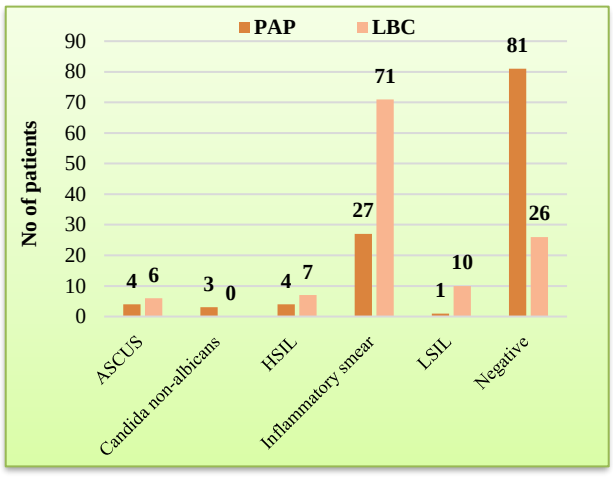

Figure 1: Comparison of PAP smear and LBC results.

VIA/VILI was positive in 22 (18.33%) subjects and negative in 98(81.67%) subjects (Figure 2).

On liquid-based cytology 6 (5.00%) had ASCUS, 7 (5.83%) had HSIL, 71 (59.17%) had inflammatory smear, 10 (8.33%) had LSIL and 26 (21.67%) had negative. Overall 23 (19.17%) had premalignant and malignant lesions and 97 (80.83%) had non-malignant lesions (Figure 1). Cervical biopsy has shown premalignant and malignant lesions in 23 (19.17%) of the study population. Among these CIN 1,2,3 were found in 13.33%, 3.33%, 0.83% and squamous cell carcinoma in 1.67% of the subjects. Chronic cervicitis was diagnosed in 57 (47.50%) of the women and negative in 40 (33.33%) of subjects (Figure 3).

Among the diagnostic methods sensitivity was lowest for PAP smear (39.1%, 95% CI 19.18% to 59.1%), followed by VIA VILI (95.7%, 95% CI 87.32% to 100%), and highest for LBC (100.0%). All the diagnostic methods

had 100% specificity, indicating that, all the methods are highly effective in ruling out the premalignant and malignant conditions. The positive predictive value of all the screening tests was 100%.

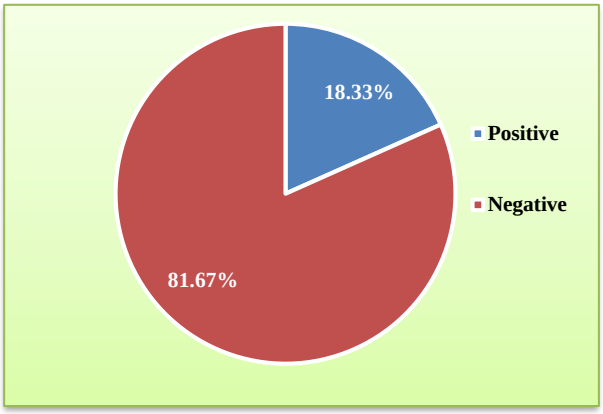

Figure 2: Results of VIA/VILI.

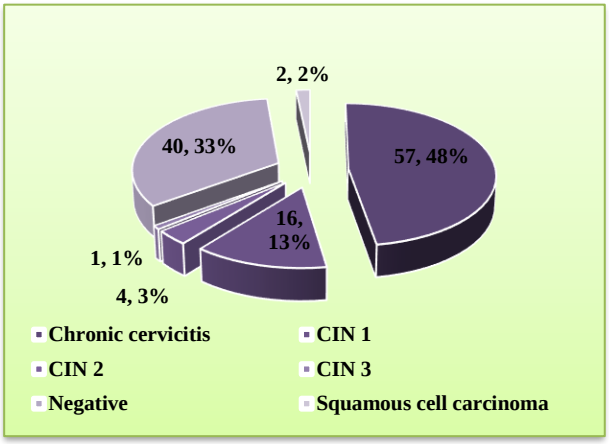

Figure 3: Cervical biopsy findings.

Table 3: Predictive accuracy.

	PAP	VIA/VILI	LBC
Sensitivity	39.10%	95.70%	100.0%
Specificity	100.00%	100.00%	100.0%
False positive rate	0.00%	0.00%	0.00%
False negative rate	60.90%	4.30%	0.00%
Positive predictive value	100.00%	100.00%	100.0%
Negative predictive value	87.40%	99.00%	100.0%
Diagnostic accuracy	88.30%	99.20%	100.0%

The negative predictive value was lowest for PAP smear (87.4%, 95% CI 81.21% to 93.6%), followed by VIA VILI (99.0%, 95% CI 96.99% to 100.0%) and highest for LBC (100%). The overall diagnostic accuracy was lowest for PAP smear (88.3%, 95% CI 82.54% to 94.1%), followed by VIA/VILLI (99.2%, 95% CI 97.54% to 100.0%) and highest for LBC (100%) (Table 3 and Figure 4).

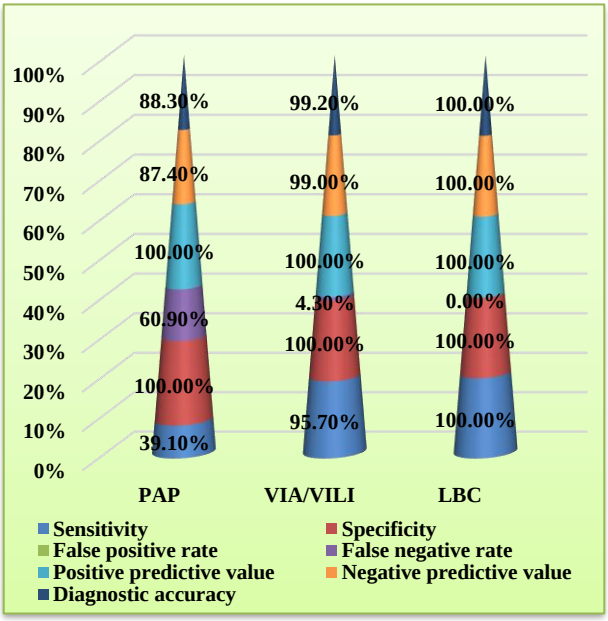

Figure 4: Predictive validity of PAP smear, VIA/VILI and LBC compared to cervical biopsy.

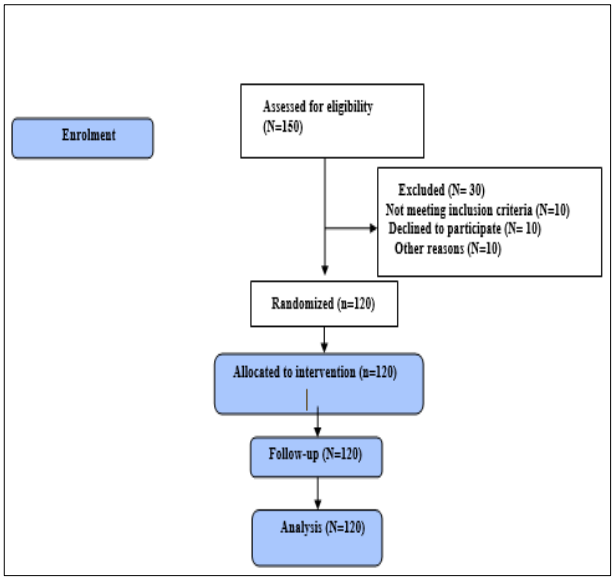

Figure 5: CONSORT 2010 flow diagram.

DISCUSSION

This study was done in 120 subjects who were recruited from women attending Gynecology out-patient and in-patient. This will favor the extensive comparison of optional tests available in this setting. Authors found that the overall specificity of all the three groups viz., conventional pap smear, VIA/VILI and LBC remained comparable whereas the sensitivity of LBC was far better

when compared to VIA/VILI and pap smear in detecting cervical pre-malignancy.

Age

Women above 35 years of age are included in present study and the mean age of present study population is 44.09 years. The mean age at marriage for this population is 18.32 years. The study conducted by Karimi MZ et al, had females who are married at a much younger age of 15.2 years and their first pregnancy was borne at 16.6 years.[8] The earlier the females are married and entering the sexual phase, there exist more chances of acquiring genital infections and the sequelae.

Parity

About three-fifths of present study participants had a parity score of two and less while only two -fifths had a score of three and above. This is in contrary when compared to the subjects who participated in a study carried out by Karabulutlu O et al, wherein more than half the participants had parity score of 3 and above.[9] According to present study, the rate of getting a pap smear done might be geared up with the decrease in parity. The possible reason could be that educated mothers will be knowledgeable of cervical cancer screening methods and also that they will be well known of birth spacing and limiting practices.

Contraceptive usage

In this study about three-fourths of the subjects underwent birth limitation methods. About one-fifth have not followed any of the contraceptive methods. Condom and OC users among the study subjects were only about 6% and 3% respectively. The positive association between OC use and cervical dysplasia was documented in a study conducted by Oh HY et al.[10] Another study provided convincing evidence that consistent condom use has improved the regression rate of cervical neoplasia.[11] In addition to these studies, Whitehouse KC et al, have exposed the risk of sterilized women in contracting progression of cervical dysplasia.[12] Usually, the women who have undergone sterilization procedure will be the major contributors of poor adherence to cervical screening follow- up. Present study, being a cross-sectional observing patient at a single point in time authors have got huge participants who had their sterilization surgeries done.

Predictive validity of Pap smear and VIA/VILI

As of now the cervical biopsy method remain more valid and hence is taken as the gold standard investigation and all other methods, viz., conventional pap smear, visual inspection with acetone, Lugol's iodine and liquid based cytology are compared against it. The sensitivity and specificity of Pap smear revealed in present study is 39.1% (CI 19.18% tp 59.1%) and 100.0%. It is 95.7% (CI 87.32% to 100%) and 100.0% respectively for VIA/VILI group. Hence the observed specificity is the same for the aforementioned tests, with a sensitivity of Pap smear being critically lesser than its counterparts. It is worth emphasizing that the results are not dissimilar with the study done by Tayyeb R et al, that showed the lower sensitivity of Pap smear and equal specificity of both the test results.[13] This in contrast to the studies by Shastri S. S et al, and Albert S et al, who observed lowered sensitivity in both these groups while Hegde D et al, observed an utterly inverse relation between the tests with pap smear showing higher sensitivity and specificity against VIA.[14-16]

In spite of the difference in sensitivity, the positive predictive value remained 100% for both the tests with the negative predictive value being slightly lower for pap smear group.

The combined effect of both conventional cytology and VIA/VILI in enhancing the results of the screening has been put forth by many studies. The meta-analysis by Qiao Let al, further intensified the finding that sensitivity of VIA/VILI got amplified when colposcopy and histology were used in combination.[17] In support of this evidence, Consul S et al, have detected a rise of sensitivity to 100% on combining pap smear with VIA/VILI thought it was perturbed by declining specificity and increasing false positivity.[18]

The diversity of the results obtained might be due to the fact that neither the groups nor their socio-demographic, immunization status (against HPV), techniques used, and the sample collected remained same from each other, with significant discrepancies in each and other factors compounded to this condition.

Predictive validity of Pap smear and liquid based cytology

The sensitivity, specificity, positive and negative predictive value of LBC is beholden to be 100%. These values are commensurately higher when compared to that produced by previous two screening tests. In corroboration to this, Cox J T et al, in a meta-analysis have found the higher sensitivity of LBC than a pap smear.[19] Likewise, work by Hussein T et al, also supports the evidence that LBC has superiority over pap smear in the detection of cervical dysplasia.[20] However, Cochand-Priollet B et al, in their study have found that conventional Pap smear has produced a considerably supreme or comparable sensitivity and specificity than LBC.[21] Though the meta-analysis performed by Ronco G et al, have backed the superiority of LBC over pap smear in diagnosing cervical pre- malignancy.[22] In contradiction to this, the meta-analysis by Arbyn M et al, have concluded that LBC remained neither sensitive nor specific in diagnosing high-grade cervical lesions on its comparison with the pap smear.[23] Fewer unsatisfactory test results have been reported in LBC by Siebers AG et

al, and also others.[24,25] Though the alterations are present between different studies, the need is to identify an appropriate screening test in a suitable setting. In countries like India that has a capacious inequity in health care delivery, with a much broader spectrum, the services have to be equipped and provided at all levels. This requires a herculean task by the Government, policymakers, inter-sectoral coordination and the people themselves in knowing and implementing all the ways that could reduce the cervical cancer burden in the near future.

CONCLUSION

Treatment decisions based on findings of the PAP smear have to be taken with caution, considering the lower sensitivity. Wherever resources are available more accurate screening methods like liquid-based cytology must be used.

Recommendations

Further large-scale studies are needed on the subjects to evaluate the diagnostic efficacy of the commonly available screening methods with respect to different benign, premalignant and malignant conditions.

Treatment decisions based on findings of the PAP smear have to be taken with caution, considering the lower sensitivity. Where ever resources are available more accurate screening methods like Liquid-based Cytology must be used.

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