

Community Based Study of Comparison between Pap Smear and Visual Inspection of Cervix with Acetic Acid and Lugol's Iodine for Detection of Malignant and Premalignant Lesions of Cervix

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ABSTRACT

BACKGROUND

Visual inspection with acetic acid (VIA) and visual inspection with Lugol's iodine (VILI) is used as a screening method in low resource setting. Introduction of such simpler and easier screening methods will have a long-term effect on the reduction of incidence of cervical carcinoma. The objective of the study was to compare the sensitivity, specificity, positive predictive value, and negative predictive value of VIA and VILI and Pap smear in screening for premalignant cervical intraepithelial lesion and subclinical early cancer cervix in the community.

METHODS

A community based prospective study involving 500 patients was done, in and around Alappuzha, and Pap smear followed by VIA and VILI were done for all patients. All positive for visual inspection cases and Pap smear, were posted for biopsy cervix and reports of both were analysed based on histopathology report. Data was analysed for finding a better tool for community screening.

RESULTS

The study found that sensitivity of visual inspection with acetic acid and Lugol's iodine was the same and was calculated as 86.7%. Positive predictive value of VIA and VILI was 44.8%. The association between VIA, VILI with Pap smear and HPR was statistically significant i.e. $p < 0.001$ for both. Sensitivity of Pap smear was 73.3%. Positive predictive value of Pap smear 40.7%.

CONCLUSIONS

Both VIA and VILI had equal sensitivity and positive predictive value, which was more when compared to Pap smear. Hence VIA and VILI can be used as a screening procedure for detecting premalignant lesions of cervix.

KEYWORDS

Visual Inspection with Acetic Acid (VIA), Visual Inspection with Lugol's Iodine (VILI), Pap Smear, Cervical Intraepithelial Neoplasia (CIN), Low Grade Squamous Intraepithelial Lesion (LSIL), High Grade Squamous Intraepithelial Lesion (HSIL), Negative for Intraepithelial Lesion or Malignancy (NILM), Histopathology Report (HPR).

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BACKGROUND

Cervical cancer is the fourth most frequent cancer in women with an estimated 570,000 new cases in 2018 representing 6.6% of all female cancers. India accounts for one-fifth of the world burden of cervical cancer.¹ Approximately 90% of deaths from cervical cancer occur in low- and middle-income countries.² There are no organized screening programmes for cervical cancer in any states of India. Data from population-based cancer registries in different regions indicate a slow, but steady, decline in the incidence of cervical cancer. But still rates remain high, particularly in rural parts of India, and the case reports have gone up due to population growth and improved health sector outreach. Awareness programmes and camps have resulted in early detection and improved survival from cervical cancer in a backward rural region. Cervical cancer is on the declining trend in India according to the population-based registries; yet it continues to be a major public health problem for women in India.² Most of our population reside in villages where cytology based screening programmes are ineffective.

Cancer cervix has been considered preventable because it has a long preinvasive state and availability of screening programmes and treatment of preinvasive lesions is effective. It has been well established that well organised screening by cytology has substantially reduced the incidence of morbidity and mortality from cervical cancer in developed countries. Many developing countries do not have ample resources to implement cytology based prevention programmes, which necessitates well organized laboratories to collect material and specialized personnel apt to render a diagnosis. There was a need to design a simple test which can be done on a community basis to detect positive cases, and hence of importance of VIA and VILI in a low resource setting

Carcinoma of cervix, has a very slow progression from pre-cancerous lesions to malignancy and since cervix is easily accessible for examination, gives us many opportunities for its early detection. It can be done as and when we get opportunity to examine patients on outpatient basis. Among all malignant tumors, cervical cancer is the one that can be most effectively controlled by screening. The well-known screening tool for carcinoma cervix is Pap smear. Newer screening techniques such as visual inspection methods and HPV-DNA testing has also demonstrated potential for early detection in many settings.^{3,4} It is also important to make the community aware of the need for further treatment and follow up once a lesion is detected, be it premalignant or malignant.

Pap smear has the disadvantage that it needs doctors for examination and good laboratory setting. visual inspection with 5% acetic acid (VIA) is a test with good sensitivity, but low specificity has been its limitation, which can lead to increased referrals and treatment of false positives, subsequently increasing the referral load as well as the cost of unnecessary treatment on the health system. Hence the challenge in screening programmes is to develop

a simple and acceptable screening method with high sensitivity and specificity.^{5,6}

The primary objective of this study was to compare the efficacy of Pap smear and visual inspection positives and to evaluate its utility in a low resource setting in screening for premalignant and malignant lesions of cervix. Secondary objective was to detect associated risk factors in the community predisposing to precancerous conditions and invasive cancer of cervix.

METHODS

This study design was 'diagnostic test evaluation' done among 500 women, who were residing in selected municipal wards of Alappuzha district. The study was for over a period of one year. All asymptomatic women aged more than 30 years were screened. Women with previous history of cervical cancer treatment, women who were pregnant, women who have obvious lesion in the cervix or frank invasive cancer cervix, women with bleeding PV, discharge PV at the time of examination, women who had a Pap smear done within one year, women who have undergone hysterectomy, women under 30 years of age and women who were not sexually active were excluded from the study.

Camps were conducted in association with PHC or social welfare clubs in selected wards of Alappuzha municipal area. Women between 30 - 70yrs were invited to attend the screening programme. In camps, following a detailed history, each patient was subjected to per speculum examination, and those patients who satisfied the criteria of study had a Pap smear taken initially followed by visual inspection with acetic acid, by placing a cotton swab soaked with 3%-5% acetic acid applied on the cervix for one minute. After removing the swab, inspection for any acetowhite areas on the cervix. Then a cotton ball soaked in lugol's iodine is applied to the cervix for one minute. Then in the same way the cervix is inspected for mustard yellow areas, especially the previous acetowhite area. If the women had either of it or both test positive, then she was asked to attend gynecology OPD at medical college, Alappuzha, for biopsy from cervix.

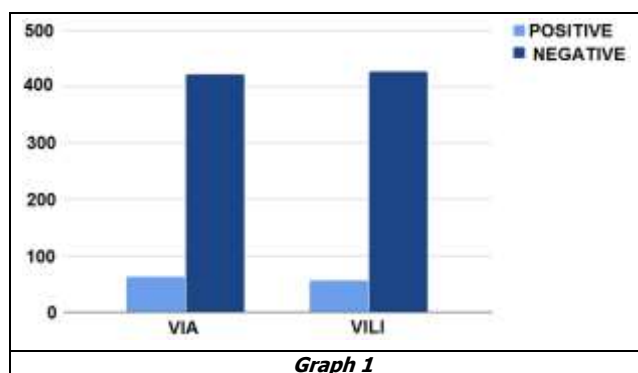
RESULTS

A total of 500 patients were screened with per speculum examination, in various camps conducted in Alappuzha municipal area, of which 483 women were selected for the study by taking into consideration the exclusion and inclusion criteria. In the study group there were 26 women (5.4%) below the age 30. There were 191 (39.5%) between 30-40 years and 161 (33.3%) were between 40-50 years. 78 (16.1%) were between 50-60 years, 24 (5%) of the subjects between 60-70 years and only 3 (0.6%) subjects were above 70 years. All the subjects were categorised based on socioeconomic status, and it was noted that 289 (59.8%) were above the poverty line and 194 (40.2%) were below

the poverty line. When divided based on parity, maximum subjects had two child birth i.e. 248 (51.3%) and 121 (25.1%) had three child birth. There were 21 subjects who had 4 deliveries, 8 (1.7%) had 5 deliveries, 4 (0.8%) had 6 deliveries and only 3 (0.6%) had 7 deliveries. Visual inspection with acetic acid were done for all 483 women of which 62 (12.8%) women were found to be positive. So a total of 421 (87.2%) were found to be negative. When visual inspection with Lugol's iodine was considered, 56 women (11.6%) were found to be positive and 427 (88.2%) were negative. Hence the positivity rate of VIA was calculated as 12.8% and the positivity rate of VILI was calculated as 11.6%.

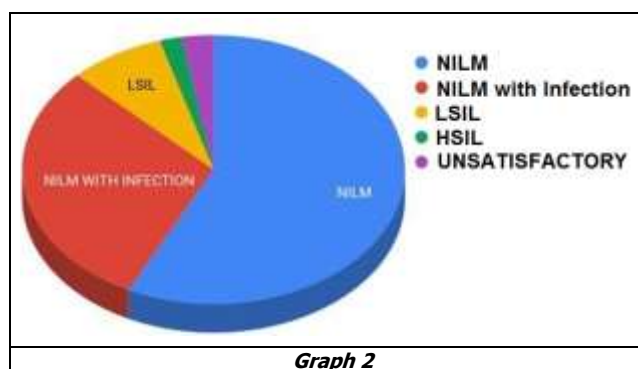
	VIA	VILI
Negative	421 (87.2%)	427 (88.2%)
Positive	62 (12.8%)	56 (11.6%)

Table 1. Distribution of Study Population on the Basis of Visual Inspection



Unsatisfactory	LSIL	HSIL	NILM with Infection	NILM	Total
13 (2.7%)	39 (8.1%)	9 (1.9%)	145 (30%)	277 (57.3%)	483

Table 2. Distribution of Study Population on the Basis of Pap Smear Report



Pap smear reports when evaluated showed that 39 (8.1%) were low grade squamous intraepithelial lesion (LSIL) and 9 (1.9%) were high grade intraepithelial lesion (HSIL). Majority of the smear reported as negative for intraepithelial lesion or malignancy (NILM) i.e., 277 (57.3%) and 145 (30%) of the smear reported to have evidence of inflammation. 13 smears were considered unsatisfactory. Hence the positivity rate of Pap smear was calculated as 9.93%. On comparing the results of Pap smear with visual inspection with acetic acid, it was noted that out of 62 VIA positives, only 19 had abnormal Pap smear i.e., 15 (38.5%)

with low grade intraepithelial lesion (LSIL) and 4(44.4%) had high grade intraepithelial lesion (HSIL). 30 cases of abnormal Pap smear had negative visual inspection with acetic acid i.e., 24 (61.5%) LSIL and 5 (55.6%) HSIL. chi-square was calculated as 37.19, df - 4, P<0.001

	NILM	NILM with e/o Infection	LSIL	HSIL	Unsatisfactory	Total
VIA negative	258 (53.4%)	122 (25.25%)	24 (4.96%)	5 (1.03%)	12 (2.48%)	421 (87.1%)
VIA positive	19 (3.9%)	23 (4.76%)	15 (3.10%)	4 (0.82%)	1 (0.2%)	62 (12.8%)
Total	277 (57.34%)	145 (3%)	39 (8.07%)	9 (1.86%)	13 (2.7%)	483

Table 3. Association of the Results of Pap Smear with Visual Inspection with Acetic Acid

Chi-square = 37.10, df - 4, P < 0.001

Similar comparison done between Pap smear and visual inspection with Lugol's iodine showed that out of 56 VILI positives, only 20 cases had abnormal Pap smear report, 16 (41%) LSIL and 5 (55.6%) HSIL. There were abnormal Pap smear reports with negative VILI, 23(59%) LSIL and 4 (44.4%) HSIL.

	NILM	NILM with e/o Infection	LSIL	HSIL	Unsatisfactory	Total
VILI negative	263 (94.9%)	125 (86.2%)	23 (59%)	4 (44.4%)	12 (92.3%)	427 (87.1%)
VILI positive	14 (5.1%)	20 (13.8%)	16 (41%)	5 (55.6%)	1 (7.7%)	56 (11.6%)
Total	277 (57.34%)	145 (3%)	39 (8.07%)	9 (1.86%)	13 (2.7%)	483

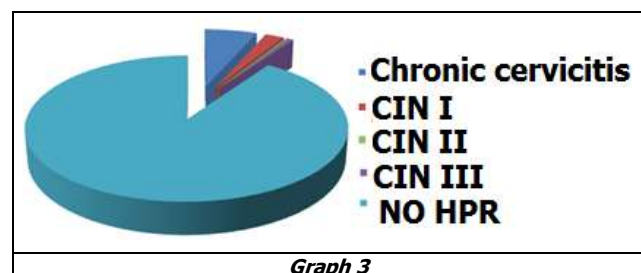
Table 4. Association of Results of Pap Smear with Visual Inspection with Lugol's Iodine

Chi square = 55.71, df - 4, P < 0.001

Women detected to be VIA, VILI positive and Pap smear report positive, were called for cervical biopsy or conization. A total of 45 biopsies were taken of which 3 cases were detected to have CIN III (0.5%). There was 1 case of CIN II (0.2%), 11 cases of CIN I (1.8%) and 30 (5%) cases of chronic cervicitis.

Chronic Cervicitis	CIN I	CIN II	CIN III	NO HPR	Total
30 (6.2%)	11 (2.3%)	1 (0.2%)	3 (0.6%)	438 (90.7%)	483

Table 5. Distribution of Study Population Based on Histopathology Report



The results of Histopathology report were compared with VIA and VILI. It was noted that 3 women with VIA positive had CIN III and 10 women with VIA positive had CIN I. 33 did not turn up. There was one case of VIA negative and LSIL on Pap smear who was detected to be having CIN II. Chi square was calculated to be 13.3 and p <

0.001. Similarly VILI and Histopathology results were also compared and it was found that maximum number of cases of VILI positive had HPR as chronic cervicitis and only 3 cases had CIN III. Chi square was calculated as 16.3 and $p < 0.001$.

	No HPR	Chronic Cervicitis	CIN I	CIN II	CIN III	Total
VIA negative	405 (83.8%)	14 (2.9%)	1 (0.2%)	1 (0.2%)	0 (0%)	421 (87.1%)
VIA positive	33 (6.83%)	16 (3.31%)	10 (2.07%)	0 (0%)	3 (0.6%)	62 (12.8%)
Total	438 (90.68%)	30 (6.21%)	11 (2.27%)	1 (0.2%)	3 (0.6%)	483

Table 6. Association of Results of VIA with Histopathology

	No HPR	Chronic Cervicitis	CIN I	CIN II	CIN III	Total
VILI negative	411 (85.1%)	13 (2.7%)	1 (0.2%)	1 (0.2%)	0 (0%)	427 (88.4%)
VILI positive	26 (5.83%)	17 (3.51%)	10 (2.07%)	0 (0%)	3 (0.6%)	56 (11.6%)
Total	437 (90.47%)	30 (6.21%)	11 (2.27%)	1 (0.2%)	3 (0.6%)	483

Table 7. Association of Results of VILI with Histopathology

The HPR positivity rate was 3.1%. Positivity rate of VIA was found to be 12.8% and positivity rate of VILI was found to be 11.6%. Pap smear positivity rate was 9.93% Of the 483 women screened positive cases were categorised as following, VIA and Pap smear positive - 19 (3.9%), VILI and Pap smear positives 21 (4.3%), VIA and VILI positives - 36 (7.4%), VIA alone positives - 6 (1.2%), Pap smear alone positives - 28 (5.3%) Of the 15 HPR positives, 9 were VIA, VILI and Pap smear positive, 4 were VIA and VILI positive and 2 were positive for only Pap smear. Those positive for all were 9. Those detected to be VIA, VILI and HPR positive but Pap smear negative was 3. Those with Pap smear and HPR positive but VIA negative was just 2 in number. Those with Pap smear positive and VIA and VILI positive with negative HPR were 6. Hence VIA detected 13 cases out of 15 biopsy proven cases, and Pap smear detected 11 cases of the total 15 cases proven by biopsy. So false positive for VIA were 16, for VILI were 17 and for Pap smear were 16.

Sensitivity of VIA	86.7%
Sensitivity of Pap smear	73.3%
Sensitivity of combined Pap smear and VIA	60%
Positive predictive value of VIA	44.8%
Positive predictive value of Pap smear	40.7%
Positive predictive value of combined Pap smear and VIA	64.3%
The agreement between VIA and Pap smear	39.6%
The agreement between VIA and HSIL	44.4%
The agreement between VILI and Pap smear	43.7%
The agreement between VILI and HSIL	55.6%

Table 8. Summary of Results of the Study

Biopsy could not be taken from some subjects detected positive for either three, as they were lost for follow up. Biopsy were not taken for negative results in the screening population. Hence specificity, negative predictive value or false positive rate could not be calculated. The results of the positive screening when compared with age group, and it was noted that the majority of VIA and VILI positives were between 30-40 years. The positivity for VIA and VILI was not significantly different across the age groups.

Taking coitarche into consideration, a maximum number of positives were noted in women who had started their sexual life before 25 years. But there was no significant association seen in this study between unhealthy cervix and coitarche. Socioeconomic status was compared with positive VIA and VILI, and it was noted that no significant association was there between the two. Parity when compared with VIA and VILI showed statistically significant association between high parity and unhealthy cervix on VILI when compared to VIA. Women of higher parity ≥ 4 was at 2.38 times more risk for ca cervix than lower parity ≤ 3 .

Age Group	VIA Positive	VIA Negative	Total
< 30	3(4.9%)	23 (5.5%)	26 (5.4%)
30 - 40	24 (38.7%)	167 (39.9%)	191 (39.5%)
40 - 50	22 (36.1%)	139 (32.9%)	161 (33.3%)
50 - 60	8 (13.1%)	70 (16.6%)	78 (16.1%)
60 - 70	5 (8.2%)	19 (4.5%)	24 (5%)
>70	0 (0%)	3 (0.7%)	3 (0.6%)

Table 9. Relation of Visual Inspection of Acetic Acid with Age Group

Chi square 2.428, $p = 0.78$

Age Group	VILI Positive	VILI Negative	Total
< 30	2 (3.6%)	24 (5.6%)	26 (5.4%)
30 - 40	23 (41.1%)	168 (39.3%)	191 (39.5%)
40 - 50	19 (34.5%)	142 (33.2%)	161 (33.3%)
50 - 60	7 (12.7%)	71 (16.6%)	78 (16.1%)
60 - 70	5 (9.1%)	19 (4.4%)	24 (5%)
>70	0 (0%)	3 (0.7%)	3 (0.6%)
Total	55 (11.38%)	428 (88.61%)	483

Table 10. Relation of Visual Inspection with Lugol's Iodine with Age Group

Chi square 3.345, $p = 0.647$

Coitarche	VIA Positive	VIA Negative	Total
<20	25 (5.17%)	170 (35.19%)	195 (40.7%)
21 - 25	30 (6.21%)	176 (36.43%)	206 (42.65%)
>25	7 (1.44%)	75 (15.5%)	82 (16.97%)
Total	62 (12.83%)	421 (87.16%)	483

Table 11. Relation of Coitarche with VIA

Chi square 1.901, $p = 0.387$

Coitarche	VILI Positive	VILI Negative	Total
<20	24 (4.96%)	170 (35.2%)	194 (40.16%)
21 - 25	26 (5.38%)	181 (37.47%)	207 (42.85%)
>25	6 (12.4%)	76 (15.73%)	82 (16.97%)
Total	56 (11.6%)	427 (88.4%)	483

Table 12. Relation of Coitarche with VILI

Chi square 1.786, $p = 0.409$

Socioeconomic Status	VIA +ve	VIA -ve	Total
BPL	29 (6%)	165 (34.16%)	194 (40.16%)
APL	33 (6.83%)	256 (53%)	289 (59.83%)
Total	62 (12.83%)	421 (87.16%)	483

Table 13. Relation of Socioeconomic Status with VIA

Chi square 1.293, $p = 0.256$

Socioeconomic Status	VILI Positive	VILI Negative	Total
BPL	25 (5.17%)	169 (3.5%)	194 (40.16%)
APL	31 (6.4%)	258 (53.41%)	289 (59.8%)
Total	56 (11.59%)	427 (88.4%)	483

Table 14. Relation of Socioeconomic Status with VILI

Chi square 0.528, $p = 0.467$

Parity	VIA Positive	VIA Negative	Total
≤ 3	28 (5.8%)	393 (81.36%)	421 (87.16%)
≥ 4	8 (1.65%)	54 (11.2%)	62 (12.83%)
Total	36 (7.45%)	447 (92.54%)	483

Table 15. Relation of Parity with VIA

Chi square 3.063, $p = 0.08$

Parity	VILI Positive	VILI Negative	Total
≤ 3	28 (57.97%)	399 (82.6%)	427 (88.4%)
≥ 4	8 (1.65%)	48 (9.93%)	56 (11.6%)
Total	36 (7.45%)	447 (92.94%)	483

Table 16. Relation of Parity with VILI
Chi square 4.287, p = 0.038, Odds ratio = 2.38

DISCUSSION

Conventional cervical cytology is the most widely used cervical screening test in the world and cytology screening programmes in several developed countries have been associated with impressive reduction in cervical cancer burden. Screening is frequently repeated at 1-5 years intervals and coverage exceeds 70% in these effective programmes. Most of the death due to cervical cancer in countries with successful cervical cytology programmes occur in women who have never been screened or in women with false negative cytology results.⁴

In this study, the sensitivity of visual inspection with acetic acid and visual inspection with Lugol's iodine was the same and was calculated as 86.7%, since all those who were VIA positive and HPR positive were also VILI positive. The positive predictive value of both VIA and VILI was 44.8%. The association between VIA, VILI and Pap smear and HPR was statistically significant i.e., $p < 0.001$ for both. The sensitivity was calculated as 73.3% and positive predictive value of Pap smear was 40.7%. So, the sensitivity and positive predictive value of both VIA and VILI was found to be more than that of Pap smear in this study. The agreement between VIA, VILI and Pap smear showed that there was more agreement between VILI and Pap smear in detecting premalignant condition, implying that VILI could be more specific than VIA. The number of false positives among VIA, VILI and Pap smear were almost similar in this study. Specificity and negative predictive value could not be calculated as biopsy cervix was not taken for all patients, which is considered as gold standard.

The sensitivity of Pap smear was found to be lower in this study group which may be due to the high prevalence of reproductive tract infections in younger group, who were the majority. This might have interfered with cervical sampling and reading of smears. Among the results NILM with inflammation accounted for about 30% of total smear reports. Hence the inflammation might have masked the underlying dysplasia, and more over women were reluctant to repeat the smear after treatment for cervicitis in order to yield more positive results in cytology.

In a study by Hossam Hassan Aly Hassan El Sakkary et al (2016),⁵ it was found that, the sensitivity of Pap smear test was 83.3%, specificity was 90.7%, positive predictive value was 50.8%, negative predictive value was 97.9% and accuracy was 90% while the VIA test had a sensitivity of 66.7%, specificity was 91%, positive predictive value was 46.1%, negative predictive value was 95.9% and accuracy was 88.5%. Via test had comparable results to Pap test regarding its sensitivity, specificity, positive predictive value, negative predictive value and accuracy. In another study by Kalgong et al., 2017,⁷ it was found in the study that,

prevalence of precancerous lesions of cervix was 12.70%. The risks factors of cervical cancer identified are age, matrimonial status, age of first sexual intercourse and parity. The association of VIA and VILI showed a sensitivity, specificity, positive and negative predictive value respectively about 93.58%; 97.01%; 82.01%, 99.04%. Hence it was concluded that, compared to PAP Smear, VIA or VILI could be used as an alternative screening method for cervical cancer in developing countries. However, histology test was recommended to use as Gold Standard to evaluate the test accuracy of VIA/VILI because it can be used to diagnose cancer, while PAP smear cannot.^{6,8}

Association of several parameters with unhealthy cervix were considered in this study, showed no association between unhealthy cervix $p = 0.78$. Hence the onset of disease could be detected in young age group itself, implicating the need for screening young women for early detection of premalignant condition. Since the precursor lesion starts much earlier, the screening at young age yields better outcomes. Age is an acceptable risk factor for the development of cervical cancer, between 45 to 70 years having the highest risk of developing and dying from cervical cancer.⁹

The age of coitarche was considered as a significant risk factor, but in this study the mean age of coitarche was 21, and no statistically significant association was noted between the two, $p = 0.387$. Being community based study, history of multiple sexual partners could not be elicited and had to rely upon the history. Hence proper association could not be calculated. In a study conducted by Pradhan, Kathmandu (2007),¹⁰ women having unhealthy cervix (69% vs. to 55%) and those with dysplasia were more likely to get married before the age of 20 years and first sexual contact before the age of 20 (68% vs. 56%) than the women with healthy cervix ($p < 0.05$). Unhealthy cervix was more commonly found in women of higher parity (≥ 3 children) than lower parity (63% vs 46%). In this study positives were noted in both the group, but significant association was noted between parity and unhealthy cervix on VILI, $p = 0.032$, the odds ratio calculated was 2.38 implying that higher parity ≥ 4 was at higher risk for carcinoma cervix.

No statistically significant association was noted between low socioeconomic status and unhealthy cervix in this study, $p = 0.256$, can be because the division between the higher and lower socio economic status was not properly demarcated. And moreover personal vaginal hygiene matters a lot in the occurrence. So screening should include both groups as the incidence in both groups were almost equal.

CONCLUSIONS

Both VIA and VILI have equal sensitivity and positive predictive value, which was more when compared to that of Pap smear; so, they can be used for screening for ca cervix. In developing countries, where adequate coverage of entire female population by cytology based screening programmes

is not feasible, VIA and VILI can be used as alternative procedures for primary screening for a large population. The advantages of visual inspection included low cost involved in screening, and availability of immediate results. Hence "see and treat" policy could be implemented, which could decrease the number of lost to follow up which can happen with Pap smear. Most of them detected to be positives were young and sexually active, hence screening programmes should concentrate on these groups in order to reduce the incidence of cancer cervix, as we can pick up early cases of premalignant lesions as well. Over treatment is of a concern as there were more false positives and other studies done have shown low specificity for VIA when compared to Pap smear, which could be computed in this study. Higher parity (≥ 4) was noted to have significant association with unhealthy cervix and higher risk (2.38 times) for ca cervix. Hence visual inspection when used in combination with Pap smear testing, colposcopy, and human papillomavirus testing, the accuracy could be increased making it a good screening modality.

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