

Accuracy of cytology, visual inspection with acetic acid or lugol's iodine in cervical cancer screening

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Abstract

Aims: To study the accuracy of visual inspection using acetic acid (VIA) or Lugol's iodine (VILI) and Pap smear for cervical cancer screening comparing with the histology from positively screened women in all three.

Method: In this descriptive study, 300 women 25 - 65 years from Gynecology clinic of T.U Teaching Hospital from December 2004 – 2005 underwent cervical screening.

Positive tests for cytology was CIN I or above, VIA was opaque aceto white lesion on applying 4% acetic acid or detection of definite yellow iodine non uptake areas with Lugol's iodine in the transformation zone or close to touching the squamocolumnar junction. Positive cases were scheduled for colposcopy directed biopsies and histological evaluation.

Results: Positive results obtained from cytology were 7.3% (22). VIA was positive in 52 women [low threshold +ve in 13.6% (41); high threshold +ve in 3.6%. (11)] VILI tested +ve in 8.7% (26).

Cervical biopsy was done in 62 women who had positive result [10 were positive with all three tests, 4 were positive with VIA and Pap smear, 14 were positive with VIA and VILI. 24 were positive with only VIA, 2 were with only VILI and 8 were positive with only Pap smear].

Histology in 19 was suggestive of CIN and carcinoma. [LSIL (12), HSIL (6), carcinoma (1)]

Pick up by Pap smear, VIA and VILI were 10; 17 and 15 missing respectively 9:[LSIL (7) HSIL (2)]; [LSIL (2)] and 4 [LSIL (3) and HSIL (1)].

VIA had highest number of false positive as compared to Pap smear and VILI.

Sensitivity for Pap smear (52.6%); low (81.1%)/high threshold VIA (80.0%) and VILI was (78.9%). Specificity for Pap smear (72 %); low was (20%) but high threshold VIA was similar to VILI (74.4%)

The positive predictive value of low or high threshold VIA, VILI and cytology were 22.0%, 72.7%, 57.7% and 45.5%; such that the compounding NPV were 80.0%, 80.0%, 88.9% 77.5%. Overall accuracy of high threshold VIA (76 %) was comparable to VILI 75.8%; cytology having 66% and low threshold VIA with 33 %.

Conclusion: High threshold VIA and VILI have higher accuracy for detection of precancerous lesions of cervix than Pap smear indicating that these test to be implicated for cancer screening which is more cost effective.

Keywords: Visual inspection; acetic acid; Lugol's iodine; VIA; VILI; screening of cervical cancer; Papanicolaou (Pap) smear.

Introduction

In the industrialized countries, cervical cancer incidence and mortality have been drastically reduced through Pap smear screening and treatment at precursor stage. Pap tests themselves have shortcomings; high

specificity in Pap smear testing cannot be achieved without reducing sensitivity. The WHO Reproductive Health Library includes summary of a meta-analysis of Pap test accuracy that reviewed 62 studies and concluded that Pap test may be unable to achieve high sensitivity and specificity concurrently¹⁻³.

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Due to the difficulties of ensuring high quality cytology based services in many settings; there have been significant interests in new approaches of screening for precancerous lesions. Of these, visual inspection of the cervix is a promising option, especially for low resource settings.

Visual inspection with acetic acid (VIA) which identifies acetowhite lesions has now been recognized as one of the promising method for cancer detection from Indian and African studies as compared to cytology under similar circumstances.⁴ Analyses from a growing number of studies in developing-country settings indicate that the sensitivity of VIA is equivalent or greater than cytology, although its specificity is somewhat lower.^{5,6}

Other forms of visual inspection with Lugol's iodine (VILI), is similar to the Schiller's iodine test used in the 1930's and has been re-evaluated in a recent studies as an alternative for use in low resource settings. Results indicate that VILI has a similar sensitivity and specificity to that of VIA (using a high-threshold cut-off), suggesting that it could be suitable alternative to cytology in low-resource settings.^{7, 8} The largest set of pooled data from multicentre study of VILI indicates that it is more sensitive than or equally specific to VIA.⁵

Many aspects of VIA and VILI make them appealing for use in low- resource settings compared to pap smear because of simpler approaches which do not require laboratory involvement. Furthermore non – physicians can perform the procedure with adequate training. The suspected precancerous lesions during the same visit can also be treated.

Aim of this study is to compare the accuracy of visual inspection using acetic acid (VIA) visual inspection using Lugol's iodine (VILI) and Papanicolau (Pap) smear as a method of cancer screening, the gold standard being histopathological report of colposcopy directed biopsy in positive cases.

Methods

A total of 300 sexually active women without history of active STD, cervical neoplasia or sign of leucoplakia in age 25 - 65 years coming to gynecological OPD from December 2004 to 2005 seeking consultation were examined using an un-lubricated bivalve Cusco's speculum. The cervix was exposed properly and excessive discharge when present was gently wiped away using a saline soaked cotton swab. Cervix was closely inspected for any macroscopic abnormalities such as cervicitis, erosion, polyp, nabothian cyst or any ulcer. The women with acute cervicitis were treated by antibiotics and were asked to come after a week. First Pap smear was taken; then visual inspection with 4% acetic acid was done and the following categorization was made.

- 1a. Low threshold VIA positive: faint, ill- defined irregular acetowhite lesions far away from the SCJ.
- 1b. High threshold VIA: Opaque, dense, well- defined acetowhite lesions touching SCJ.
2. VILI positive: Well – defined, dense, thick, bright mustard yellow or saffron yellow, iodine non uptake areas touching the SCJ.
3. Positive smears: low grade squamous intraepithelial or worse lesion by cytology.

Positive Pap smear, VIA or VILI were subjected to colposcopy directed biopsy and forwarded for histology which was reviewed by Pathologists from the Department of Pathology TUTH as was the Pap smear for cytology.

VIA testing results were categorized as negative, single positive, or double positive based on the criteria given in table After VIA, Lugol's iodine was applied to the cervix with help of cotton swab stick and naked eye examination was done. Categorization was done as negative or positive based on the criteria given in table 2.

Table1. Criteria for categorizing VIA test results

VIA test outcome	Criteria
Negative	<ul style="list-style-type: none"> · No aceto white lesion · Aceto- acetowhitening or endocervical polyps, nabothian cyst · Prominent white line-like acetowhitening of the SCJ
Single positive	<ul style="list-style-type: none"> · Faint, translucent, ill-defined irregular acetowhite lesion · Definite, angular, geographic acetowhite lesion far away from the SCJ.
Double positive	<ul style="list-style-type: none"> · Opaque, dense, dull, definite well-defined acetowhite lesion touching the SCJ or close to the external os. · Large, circumferential, well-defined, thick dense aceto white lesions. · Growth on the cervix turns acetowhite.

Table 2. Criteria for categorizing VILI test results

Results	Criteria
Negative	<ul style="list-style-type: none"> • Normal cervix where SCJ turns Mahogany brown or black and the columnar epithelium does not change color. • Patchy, indistinct, ill defined, color less, or partially brown areas in transformation zone. • Scattered, irregular, ill-defined non- iodine uptake Ares on cervix. • Thin, yellow, non-iodine uptake areas with angular or digitizing margins, resembling geographical areas located far away from SCJ.
Positive	<ul style="list-style-type: none"> • Well-defined, dense, thick, bright mustard yellow or saffron yellow, iodine non uptake areas touching the SCJ. • Circumferential, well defined, thick dense, yellow lesion occupying large portion of cervix. • Growth on cervix turns yellow

VIA and VILI positive women were subjected to colposcopy directed cervical biopsy as had been done for Shiller’s positive areas in VILI or abnormal looking areas in acetowhite areas for VIA at the same sitting. But for pap smear positive cases colposcopy directed biopsy was done in the next visit after seeing the results.

Results

Only 1.5% of the total women attending Gynaecology OPD, underwent the tests of which 96.3% were married, majority being multiparous, who came with complaints of lower abdominal pain (81%), vaginal discharge 62%, back ache (47%), intermenstrual bleeding (26.7%), dyspareunia (11.0%) post coital bleeding (4.3%) or former history of treatment for STD (2.3%). There were 56.6% in the age group of 20-29, 40.8% in 30-93 and 2% were in 60-69 years. Ninety one percent were in the reproductive age while 39% were post menopausal.

Cervix was healthy in 39.3% and unhealthy in 60.7%.

On Pap smear 22/300 (7.3%) tested positive: 3 had HSIL and 19 had LSIL. Among 278 negative cases, 272 were normal or inflammatory 6 were atypical cells of undetermined significance.

Regarding VIA, 52/ 300 women had acetowhite lesion: single positive (41) and double positive (11). The proportion of women screened positive with low threshold VIA in the study was 13.6% and with high threshold VIA is 3.6%.

On VILI, 26/ 300 (8.7 %) had absence of iodine no uptake areas.

Ten women were positive with all three tests; 4 were positive with VIA and PAP smear; 14 were positive

with VIA and VILI; 24 were positive with only VIA, 2 only with VILI and 8 were positive only with Pap smear.(table 3). Colposcopy directed biopsy was performed in 62 (20.7%) tested positive cases.

Out of 62 women undergoing biopsy 19 (6.3%) was positive with CIN (LSIL 12, HSIL 6, carcinoma 1).

Individually, Pap smear was positive in 22 and 10 were biopsy proven, thus showing a sensitivity of 52.6% (C.I 29.5, 74.8) and specificity of 72.1% (C.I 56.1, 84.2) for cytology.

On the other hand 40 cases were Pap smear negative but VIA/VILI positive who underwent biopsy where 9 cases had positive result on biopsy meaning cytology missed 9 cases. Hence PPV was 45.5% (C.I25.1, 67.3) or NPV as 77.5% (C.I 61.1, 88.6).The accuracy of Pap smear was 66 %.

VIA was positive in 52/300 of whom 41 had single positive while 11 had double positive. Of these 41 single positive cases 9 were found positive on biopsy and remaining 32 had benign histological changes.

Table 3. Test outcome

Test outcome	Number
Only VIA	24
Only VILI	2
Only pap	8
VIA+VILI	14
VIA + PAP	4
VILI + PAP	0
VIA+ VILI + PAP	10
Total	62

Of the 11 double positive specimens evaluated, 8 were found positive on biopsy and remaining 3 had benign histological changes. This indicated a sensitivity of 81.8 (C.I 47.8-96.8%) and specificity of 20.0. % (C.I 9.6-36.1) for low threshold VIA(+). The sensitivity of 80.0% (C.I 44.2; 96.5) and specificity of 72.7% (C.I 39.3, 92.7) for high threshold VIA(++).

On the contrary 10 cases showed negative VIA but had positive Pap smear /VILI and biopsy findings. So, VIA missed 2 biopsy positive cases. PPV of low threshold VIA (+) was 22.0 % (C.I 11.1, 38.0) and for high threshold VIA was 72.7% (C.I 39.3-92.7). NPV for low threshold VIA and high threshold VIA were the same, being 80.0% (C.I 44.2, 96.5). The accuracy of low threshold and high threshold VIA was found to be 33.3% and 76.2% respectively.

VILI was positive in 26/ 300; 15 were found positive on biopsy and the remaining 11 had benign histological changes. This indicated a sensitivity of 78.9% (C.I 53.9, 93.0) and specificity of 74.4% (C.I 58.5, 86.0) for VILI.

Thirty-six cases were negative for VILI but were positive for Pap smear/VIA that were biopsied. Of these VILI missed 4 cases that were positive on biopsy. (Table 4). Out of the total 19 biopsy proven true positive cases VILI picked up 15 making PPV of VILI as 57.7% (C.I 37.2-76.0) and NPV as 88.9% (C.I 73.0, 96.4). The accuracy of VILI calculated was 75.8%.

Of the 62 biopsy 19 had histological changes [2 /5 menopausal women had significant findings.

Comparison of result of VIA, VILI and Pap smear

Sensitivity of low threshold VIA was 81.1% which was similar to high threshold VIA (80.0%). The sensitivity of VILI was 78.9% while pap smear had lower sensitivity of 52.6%.

The specificity of low threshold VIA was 20 % while using high threshold specificity it came to be 72.7% which was similar to Pap smear 72.1%. VILI had higher specificity of 74.4% with PPV of 22 % in low threshold VIA while it was 72.7% with high threshold VIA. PPV of VILI and pap came to be 57.7% and 45.5%

respectively. NPV was higher for VILI 88.9% while it was same for both low and high threshold VIA (80.0%). Pap smear had NPV of 77.5%. High threshold VIA had higher accuracy rate (76.2%) which was similar to VILI, 75.8%. Low threshold VIA had accuracy rate of 33.3% and accuracy rate of Pap smear was 66.1%.

Discussion

The result of current study indicates that VIA and VILI are simple objective tests. The result of this procedure is available immediately, allowing an algorithm of further investigations to be carried out for the identification of cervical lesions. VIA and VILI may find a place as an alternative low technology and low cost method of screening and case finding. It would be inappropriate to compare our small findings with other studies involving large population in series of trials; still this has been necessary as a part of study.

In studies done in rural India VIA positivity ranged from 3- 4% to 9.8%.^{9,10}

The sensitivity and specificity for cytology in the present study were 52.6% and 72.1% respectively as compared to HS Cronje with sensitivity of 19.3% and specificity of 99.3%.¹¹ While it was 44.3% and 90.6% respectively in a study by Gaffikin et al.¹²

In fact acetic acid study has been promising in African and Chinese populations.^{13,14}

The specificity for cytology in this study was 72 % which was comparable with that of others.^{7,8,11,12}

Unlike ours, the pathologists in these studies were given a review course designed to standardize their skills in cytology before the assignment and 10% of random sample were forwarded to another cytopathologist (JHPIEGO).¹²

In the meta- analysis of 62 studies of cytology conducted between 1984 to1992 the mean sensitivity was found to be 58% (range 11-99%) and mean specificity was 68% and 71. %^{3,12}

High number of false positive and consequently low specificity for low threshold VIA could be due to the

Table 4. Biopsy result

Histopathology	Number	Percent
Normal/Cervicitis	43	69.4
LSIL	12	19.4
HSIL	6	9.7
Carcinoma	1	1.6
Total	62	100

Table 5. Comparison of VIA, VILI, Pap smear

	Pap smear	VIA +	VIA ++	VILI
Positive screen	7.3%	13.6%	3.6%	8.7%
Sensitivity	52.6%	81.1%	80.0%	78.9%
95% C.I.	(29.5, 74.8)	(47.8, 96.8)	(44.2, 96.5)	(53.9, 93.0)
Specificity	72.1%	20.0%	72.7%	74.4%
95% C.I.	(56.1, 84.2)	(9.8, 36.1)	(39.3, 92.7)	(58.5, 86.0)
PPV	45.5%	22.0%	72.7%	57.7%
95% C.I.	(25.1, 67.3)	(11.1, 38.0)	(39.3, 92.7)	(37.2, 76.0)
NPV	77.5%	80.0%	80.0%	88.9%
95% C.I.	(61.1, 88.6)	(44.2, 96.5)	(44.2, 96.5)	(73.0, 96.4)
Accuracy--	66.1%	33.3%	76.2%	75.8%

large number of inflammatory lesions.^{15,16} However the specificity of high threshold of VIA was higher (72.7%). The low VIA positivity in menopausal women may be due to the movement of transition zone and the squamocolumnar junction into the cervical canal.

With regards to VIA, VILI and cytology the results were consistent with recent studies findings of India and Africa which have shown that VIA, VILI are more sensitive than cytology.

VIA was more sensitive than Pap smear for the detection of pre-invasive and invasive stage of cervical carcinoma as in other study.¹²

Sankarnarayanan⁸ et al reported equally comparable specificities for both high thresholds VIA (86.5%) and Pap smear (87.8%). The specificity of low threshold VIA was lower (78.0%) but the difference was not significant. Study by Sankarnarayanan⁹ et al reported equally comparable specificities for both tests, 92.2% for VIA and 92.7% for cytology which is not similar to our study.

NPV in both low threshold VIA and high threshold VIA was similar (80.0%). While it was higher for VILI (88.9%) but lower for Pap smear (77.5%). The high NPV of both VIA and VILI warrants particular mention. The use of VIA and VILI as a primary screening test means that women assessed as test negative would be reassured that most probably they do not have HGSIL or cancer. The results in this study have been found consistent with the study done by JHPIEGO^{8,12}

To sum up, low threshold VIA showed accuracy of 33.3% while high threshold VIA showed accuracy of 76.2%. Accuracy rate was also higher for VILI (75.8%) compared to Pap smear which was 66.1%. However the difference was not statistically significant. Since the reference test of biopsy was performed on screened

positive only, we could not obtain an accurate estimate of the prevalence of the disease. This is the limitation of this study.

Conclusion

In women undergoing screening for pre- invasive and invasive cervical cancer high threshold VIA and VILI was found to be more sensitive, more specific compared to Pap smear. Low threshold VIA was more sensitive but was less specific than Pap smear, resulting high false positive results. High threshold VIA could be used to improve specificity without loss in sensitivity. Thus high threshold VIA and VILI can be combined as a primary screening method for cervical cancer to obtain an increased effectiveness while being safe, simple and cost effective providing results instantly.

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