Original Article

Evaluation of adjunctive tests for cervical cancer screening in low resource settings

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Abstract

BACKGROUND: Visual inspection of cervix after application of acetic acid (VIA) is an effective screening tool for cervical cancer in low resource settings, but its low specificity leads to high referral rates. Adjunctive testing may overcome this drawback. **AIMS:** This pilot study was aimed to assess test performances of VIA, human papillomavirus (HPV) testing and Pap smear, individually and in simulated combinations, to determine the probable best screening option. **SETTING AND DESIGN:** Gynecology outpatient department (OPD); cross-sectional study. **MATERIALS AND METHODS:** One hundred women with complaints of irregular vaginal bleeding or discharge, post coital bleeding or unhealthy cervix on examination underwent Pap smear, HPV testing, VIA, colposcopy and biopsy, if indicated, in this screening order. **STATISTICAL ANALYSIS:** Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each of the tests with a biopsy result of ≥HSIL taken as the gold standard. Simulated parallel and sequential combinations for VIA/Pap, VIA/HPV and HPV/Pap were calculated and compared with individual test performance. **RESULTS:** Prevalence of abnormal Pap smears was 5%, VIA positive 51% and HPV positive 16%. Sensitivity and specificity of VIA were 100% and 53.3% respectively. For HPV and Pap tests corresponding figures were 85.7%, 89.7% and 50%, 98.9% respectively. The best simulated combination with a balance of sensitivity and specificity was of VIA followed by HPV testing (sensitivity 85.7%, specificity 95.4%). **CONCLUSION:** Addition of HPV testing to VIA can increase the specificity of VIA, thereby reducing the referral rates without compromising the sensitivity of the test.

Key words: Adjunctive testing, cervical cancer, human papillomavirus, screening, VIA

Introduction

Each year approximately 470,000 new cases of invasive cervical carcinoma are diagnosed worldwide, of which 230,000 women die from it.[1] In India, it is estimated that 126,000 new cases occur every year.[2] The conventional method of screening by cervical cytology requires repeated testing and a relatively sophisticated infrastructure. Therefore, alternative methods, such as visual inspection after application of acetic acid (VIA) and human papillomavirus (HPV) DNA testing have been developed.[3,4] Studies on VIA show high sensitivity but low specificity, leading to high referral rates.[5,6] Adjunctive testing using two tests in parallel or sequential combination improves specificity without compromising sensitivity.[7] We conducted a pilot study where women at high risk of developing cervical cancer were screened using conventional cytology, HPV testing, VIA and colposcopy. The joint test qualities of various simulated combinations of the tests were studied and compared to individual tests.

Materials and Methods

Study population

This cross-sectional study was carried out in the Gynecology OPD from January through April 2003. Women with complaints of persistent vaginal discharge,

intermenstrual bleeding, post coital bleeding or those found to have an unhealthy cervix on examination were invited to participate in a cancer screening programme. Exclusion criteria were age <30 years; unmarried; hysterectomized; prior surgical procedures on cervix; gross tumor on cervix; and pregnancy. Informed written consent was taken from the women, informing them of the background of the study, risks and benefits and voluntary nature of participation. Ethical clearance was obtained from the institutional ethical committee.

Clinical examination

An oral questionnaire was administered to all women pertaining to age, parity, socio-economic status,[8] smoking and contraception history. A nurse was trained to perform VIA of the cervix. Training consisted of didactic teaching on the anatomy and pathophysiology of the cervix, extensive review of photographs of normal and abnormal cervices and supervised hands-on clinical examination.

The examination included sequentially:

1. A Pap smear obtained by a gynecologist using an Ayre's spatula and a cytobrush.
2. A cervical sample for HPV DNA testing collected by a gynecologist into an HPV specimen collection tube (Digene Corporation, Gaithersburg, MD).
3. VIA of the cervix after application of 5% acetic acid performed by the nurse. The results were interpreted using the criteria laid down by the International Agency for Cancer Research (IARC).[9]
4. Colposcopy and guided biopsies performed by a second gynecologist blinded to the findings of the previous tests.

Laboratory tests

HPV DNA was assayed using the Hybrid Capture 2™ (HC2) HPV DNA assay (Digene Corporation, Gaithersburg, USA), performed using the microwell format and probes for "high oncogenic risk" HPV types (i.e, types 16,18,31,33,35,39,45,51,52,56,58,59,68).[10] Assay results are reported as relative light units (RLU) of HPV DNA in the sample. A sample is considered to be positive if the ratio of the sample RLU to the positive control RLU is ≥1.0. Colposcopic lesions were graded using the Reid Colposcopic Index.[11] Endocervical curettage was performed if no lesions were visible. Biopsy was carried out for lesions on colposcopy with a Reid score of ≥0. The histologic results were reported using the three-tier CIN classification system. Pap smears were reported using the Bethesda system terminology and results were available within two weeks of the Pap smear being taken.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of each test were calculated. Biopsy results or the colposcopy results where biopsy was not taken in case of no lesion, were used as the reference standard for measuring the true disease, thereby adjusting for verification bias. The threshold defining disease was the presence of any lesion >CIN1. The criteria for being considered test positive for the three tests were as follows: for Pap smear, a finding of LSIL and above; for HPV, positive RLU of >1.0; for VIA, finding of a well-defined aceto-white area arising from the transformation zone or well defined white streaks on the columnar epithelium.

To evaluate the benefits of adjunctive testing, net sensitivity and specificity of various screening combinations were applied to the original data. With this approach, two tests were applied either in parallel (i.e. both tests are performed for all women and if either was positive, the result was taken as positive) or sequentially (i.e. second test was performed for only those women assessed positive on the first test). While the former approach reduces the false negative rate, the latter reduces the false positive rate. Net sensitivity and specificity values represent the joint probability of disease/non-disease being accurately detected when using more than one test.

Results

One hundred women were enrolled in the study. The demographic characteristics of the women are presented in Table 1. The presenting complaint was vaginal discharge in 80 women, irregular vaginal bleeding in 13 women and post coital bleeding in one woman. Unhealthy cervix was seen on speculum examination in 38 women.

Results of VIA, Pap smear, colposcopy and biopsy, if indicated, were available for all 100 cases. HPV results were available for 94 patients. Biopsy was taken in 60 patients. There were two cases of CIN 1, three cases of CIN 2, three cases of CIN 3 and two cases of invasive squamous cell carcinoma. The prevalence of biopsy-confirmed high-grade and low-grade SIL did not differ within the different age groups: three cases of HSIL and one of LSIL were found in the age group of <40 years as well as in the age group ≥40 years. Both the women with invasive carcinoma were older than 45 years. However, the majority (9/15) of HPV positive women belonged to the age group of <40 years.

A high proportion of women (51%) were classified by the nurse as having an abnormal result when screened

Table 1: Socio demographic profile

Characteristic(s)	Number of women (n=100)
Age (years)	
Mean ± SD	38.04 ± 8.00
Median (range)	36 (30-74)
Age at first coitus (years)	
Mean ± SD	19 ± 3.31
Parity	
0-3	67%
≥4	33%
Education	
No schooling	35%
Primary school	13%
High school	28%
>High school	24%
Socio-economic status	
Low and low upper	46%
Middle and upper middle	50%
Upper	4%
H/o tobacco exposure	7%
H/o ever-use of oral contraception	6%

by VIA after the application of a 5% solution of acetic acid. All eight women with high-grade disease or cancer were identified as VIA positive.

Colposcopic diagnosis of premalignant and malignant lesions, based on Reid index correlated well with the histologic diagnosis. Only in one case of invasive carcinoma, the Reid score was <2, however VIA was positive in this case. In the remaining seven cases of biopsy proven lesions ≥HSIL, the Reid index was ≥3. One case of LSIL (CIN 1) with a Reid score of 1 was missed by VIA. Among the 23 women with false positive VIA results, nine women had RCI ≥3, six had RCI 0-2 and eight women had no colposcopically evident lesion.

Of 100 Pap smears taken, the overall abnormal rate was 10%, including ASCUS, SIL and invasive carcinoma. A diagnosis of ≥LSIL/carcinoma was made on 5% of all Pap smears. Of the eight biopsy-confirmed cases of ≥HSIL, Pap smear could diagnose only four (50%). One woman with carcinoma had atypical cells in the Pap smear. On the other hand, 80% of women in whom Pap smear was classified as SIL or invasive carcinoma had biopsy confirmed high-grade SIL (CIN 2,3) or invasive cervical carcinoma. Pap test sensitivity improved when the cut-off was used as ≥ASCUS rather than LSIL (62.5% versus 50%).

Using the Hybrid Capture II HPV DNA assay, 15 out of 94 women (16%) were found to be high risk HPV DNA positive. High risk HPV DNA was identified in 89.7% of the women with biopsy confirmed high grade SIL (CIN 2,3) or invasive cancer and in 10% of cases without disease.

The sensitivity, specificity, PPV and NPV of the individual tests are presented in Table 2. The results for sequential testing scenarios are presented in Table 3. Consistent with the nature of sequential testing, the hypothetical net specificity in all schemes involving VIA was higher than the value observed for VIA as a stand-alone test. The simulated test combination involving VIA and HPV demonstrated the best balance of sensitivity and specificity (87.7% and 95.4%).

The simulated parallel combination that resulted in the highest net sensitivity was that of VIA/HPV and VIA/Pap (both 100%). However, the net specificity was only 45.9% and 52.2% respectively. HPV and Pap demonstrated the best balance of sensitivity and specificity (87.5% and 89.1%).

Discussion

Cervical cancer accounts for the highest number of deaths from cancer among women in India. Although the value of repeated Pap smears in screening for this disease and its precursors has long been established in the West, it is clear that logistic requirements cannot be met in developing countries in the foreseeable future. Alternative methods for low resource settings such as VIA by trained paramedical workers offer hope for universal screening. The sensitivity of VIA ranges from 71-77%, comparing well with Pap, but specificity is 64-80%.[5,12-14] At a programmatic level, this implies a large number of unnecessary referrals with varied logistic problems. In settings where the prevalence of cervical disease is low, VIA and VILI may not perform as well as stand-alone tests.[15] These techniques need careful monitoring for quality control.[16]

Adjunctive testing is one way of improving specificity of the test without compromising sensitivity.[7] The discovery of the role of HPV in the causation of CIN and cervical cancer led to the development of new tests, e.g. Hybrid Capture, a simple commercial test that could be combined with VIA to improve specificity. Although the cost of HPV testing is presently high,

Table 2: Overall test performance of the individual tests with relation to the gold standard (biopsy positive for ≥ HSIL or invasive carcinoma)

Gold		TP†	TN†	Sens‡	Spec‡	PPV‡	NPV‡	DA‡
Pos	Neg							
VIA by Nurse								
8	92	8	49	100	53.3 (42.8-63.7)	15.7 (7-28.5)	100 (46.7-66.8)	58
HPV DNA testing								
7	87	6 (42.1-99.6)	78	85.7 (81.2- 95.1)	89.7 (16.3-67.7)	40.0 (93.1-99.9)	98.7 (78.7-90.5)	84
Pap > ASCUS								
8	92	5 (24.5-91.5)	87	62.5 (87.7-98.2)	94.7 (18.7-81.3)	50.0 (90.6-99.3)	96.07 (84.8-96.4)	92
Pap > LSIL								
8	92	4 (15.7-84.3)	91	50.0 (94.1- 99.9)	98.9 (28.3-99.4)	80.0 (89.6-98.6)	95.8 (88.7-98.3)	95

*Values in brackets are 95% confidence intervals. †TP = True positive, TN = True negative, FP = False positive, FN = False negative. ‡Sens (sensitivity) = (TP/TP+FN) x 100; Spec (specificity) = (TN/TN+FP) x 100; PPV (positive predictive value) = (TP/TP+FP) x 100; NPV (negative predictive value) = (TN/TN+FN) x 100; DA (diagnostic accuracy) = (TP+TN/TP+FP+TN+FN) x 100.

Table 3: Measures associated with sequential testing and parallel testing (n=94)

Test	VIA/Pap (95% CI)		VIA/HPV (95% CI)		HPV/Pap (95% CI)	
	Sequential	Parallel	Sequential	Parallel	Sequential	Parallel
Net sensitivity	50% (15.7-84.3)	100%	85.7% (42.1-99.6)	100% (8.5-75.5)	37.5% (47.3-99.7)	87.5%
Net specificity	100%	52.17% (41.5-62.7)	95.40% (88.6-98.7)	45.98% (35.2-57.0)	100%	89.1% (80.9-94.7)
Net PPV	100%	15.38% (6.9-28.0)	60% (26.2-87.8)	12.9% (5.4-24.9)	100%	41.2% (18.4-67.1)
Net NPV	95.8% (89.7-98.8)	100%	98.8% (93.5-98.9)	100% (88.3-98.3)	94.9% (93.4-99.9)	98.8%
Diagnostic accuracy	96% (89.9-98.9)	56% (45.7-65.9)	89% (81.2-94.3)	47% (36.9-57.2)	95% (88.7-98.3)	89% (94.5-99.9)

HPV - Human papillomavirus

efforts are on worldwide to develop cheaper tests for at least the commonest high-risk HPV types and it is hoped that these will be a reality by 2008.

In this pilot study, the joint test qualities of various simulated combinations of the screening tests were compared to the qualities of each test as a stand-alone screening tool and the advantages and disadvantages of adjunctive testing were considered in the context of a low resource setting. We have provided performance measures for various simulated combinations of VIA, cytology and HPV, sequentially and in parallel, as a means of identifying the most appropriate approach to cervical cancer screening in a low resource setting. All women enrolled in the study underwent the reference standard test, thereby allowing estimation of direct measures of individual and net test qualities, unaffected by verification bias. Testing schemes for which results are not immediately available, especially in less developed countries, result in unacceptably high rates of loss to follow up among the tested population.[17] If any of the tests in the combined scheme could provide immediate results, then using that test first would lead to lower overall lost-to-follow up rates than if all tests were associated with a processing or a reporting delay.

VIA offers this advantage: using VIA as the first of two possible tests means there is potential for the second test to be performed at the same clinic visit. HC2 is a robust, simple test, which does not require sophisticated laboratory equipment or personnel. Already the rapid

HPV test is being tested in field trials.

Assuming that detecting cases of disease is more crucial to a screening programme as compared to accurate identification of non-cases, our results suggest that sequential testing has a better diagnostic accuracy than parallel testing. VIA followed by HPV testing would be the most effective screening approach to identify women who need further management. Similar conclusions were drawn from the study of Blumenthal *et al*.[18]

Pap smear and HPV testing in parallel had a diagnostic accuracy of 89%. The advantage of this combination, which is currently being propagated in the West, lies in its strong negative predictive value, which can decrease the frequency and closeness of follow-up required. Women who are Pap and HPV negative are at extremely low probability of developing disease, while those who are positive need more frequent evaluation, aided by colposcopy.

The "see-and-treat" approach has been proposed as a good strategy in developing countries for management of cervical pre-cancers.[19,20] Although VIA as a stand-alone screening test performs well in terms of detecting true cases of disease, the false positive rate was 46.7% (43/92) in the present study, indicating that for every one case, five women would be over-treated. The combination of VIA followed by HPV testing could more accurately identify non-disease cases, at the expense of only modest reductions in sensitivity and detection rates. This approach will reduce the referral and treatment rates as well as the number of visits required for diagnosing the disease. The results from this pilot study are encouraging and suggest that larger multicentric studies would help to guide national policy.

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