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Original Research Article

Comparative Evaluation of Results of Pap Smears and HPV Hybrid Capture 2 Performed On Cervical Samples Before and After Application of Acetic Acid

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ABSTRACT

Aim: To assess the feasibility of triaging VIA (Visual inspection with Acetic Acid) positive women with a test with higher specificity, either Pap or HPV DNA test (Human Papilloma Virus) in the same sitting at community level. This strategy if successful shall prevent unnecessary referrals to tertiary care in resource constrained settings and also increase the programmatic yield.
Objective: To investigate, whether the 5% acetic acid, used in VIA screening will compromise the cellularity of Pap test or results of HPV DNA test.
Materials/ Methods: Fifty women were randomised to either HPV test arm or Cytology test arm. The 25 women in HPV DNA arm underwent a HPV DNA HC2 test before and five minutes after application of 5% acetic acid. In cytology arm 25 women underwent Pap test before and five minutes after application of 5% acetic acid.
Conclusions: According to results of this study, 5% acetic acid affected the cellularity of cytology smears and may affect interpretation of dysplastic smears. HPV DNA test results remained unaffected by acetic acid. These results suggest that HPV HC2 may perhaps be a better triage test for VIA positive women. But this needs confirmation with a larger sample size. These findings are extremely crucial in the scenario that National Cancer Control Programme in many low-income countries including India are adopting VIA based screening and there is unavailability of resources to handle the large number of false positives emerging from positive VIA test.

Key words: Acetic acid, Pap smear, HPV DNA test.

INTRODUCTION

Though cervical cancer has a preventable precancerous stage that can be diagnosed by simple and effective screening methods, cervical cancer still remains third common cancer in women globally and is the second most common cancer among Indian women. [1]

In order to reduce the cervical cancer burden, it is important to screen the women and detect the cervical cancers in pre-cancerous stage when complete cure is still possible. [2]

Pap smear has decreased the cervical cancer mortality among developed countries, [3,4] but in Low Middle Income Countries (LMIC) similar results are not evident for various reasons. It is difficult to implement cytology-based screening programme in LMIC mainly due to issues of

lack of awareness among women, uneven distribution of health care facilities, need of infrastructure in terms of laboratory, a trained cytologist to interpret the test reports with good quality control measures in place and trained staff to conduct the test**.** [5,6]

Pap test has a suboptimal sensitivity, but has a good specificity. [7,8] The robust study from India has shown that single round of HPV DNA (Human Papilloma Virus) screening test to significantly reduce the cervical cancer mortality. [9] But HPV based screening programs in majority of the LMICs still remains a challenge due to logistics difficulty, cost and moderate sensitivity of the test in Indian setting. [10]

Amongst the various available methods for cervical cancer screening, screening with VIA (Visual Inspection with Acid acid) appears the most feasible and effective option for LMICs, with advantages of results being available immediately and the test showing good performance even with a trained non-clinician provider. Immediate availability of the screening test results makes it convenient to triage a woman with another screening test or treat the women in the same sitting. The issue with screening programs with VIA is its high false positivity test results, leading to increased burden for further diagnostic investigations in the health care system. [11-14]

Currently at community screening level the women are referred to the secondary health care centres for further work up. Health is usually not a priority for women in developing countries and usually they are less aware of their health issues. This process of referral of screen positives leads to high rates of non-compliance for further diagnosis & management making a screening program ineffective.

So, a good strategy would be, to triage a VIA positive women on primary screening with Pap test or HPV DNA test, which have good specificity, in the same sitting without much waiting period so as to reduce the referral burden for further investigations, unnecessary treatment and also loss to follow up. The correct execution of the triage test is crucial as improper execution might result in false diagnosis.

For the correct execution of the above strategy, it is crucial that application of 5% acetic acid, which is used for VIA screening should not affect the results of the secondary triage tests (Pap/ HPV DNA test), if done in the same sitting at a short interval of 5 minutes when women is still on the examination table.

The current study was undertaken with the objectives of assessing the feasibility of triaging VIA positive women (on primary screening), with either Pap or HPV DNA test (these tests have higher specificity) immediately at an interval of 5 minutes in the same sitting and investigating, whether the 5% acetic acid, used in VIA screening compromises the cellularity of Pap test or results of HPV DNA test. The study was initiated after obtaining Ethics Committee approval and registering with Clinical Trial.gov.

MATERIALS AND METHODS

The present study is a Pilot cross-sectional study approved by Institutional ethics Committee, Tata Memorial Hospital Mumbai, and is registered at clinical trials.gov (NCT02363244).

Inclusion criteria

Sexually active non-pregnant women in the age group of 30-60 years attending the Preventive Oncology screening OPD were enrolled after obtaining inform consent.

Exclusion criteria

Women with prior history of hysterectomy or prior history of cervical precancerous or cervical cancer were excluded from the study.

Procedure of enrollment

Convenience samples of 50 eligible married women in the age group of 30-60 years were enrolled after obtaining informed consent. Trained clinical researcher administered the informed consent form in the vernacular language to the study participants in the presence of a witness. For

those unable to read, the entire informed consent was read out in the vernacular language and explained the contents of the informed consent form in the presence of impartial witness.

The informed consent clearly described the purpose, objectives and the process of the study. Participants were made aware of their right to refuse to participate. They were made to understand the confidentiality that would be maintained in the study. Any doubts that arised from information sheet were answered by the treating doctors. Sufficient time was given to each participant to think upon thereafter. Personal interviews were conducted to record the sociodemographic data, reproductive and medical history information by the treating doctors.

Women were randomly allocated to HPV DNA arm OR Cytology arm using lottery method (where in 25 chits with HPV and 25 chits with cytology written inside had been placed). The allotment to either HPV or cytology arm depended on the test mentioned in the chit. For the women allotted to cytology arm, conventional Pap smear was collected before and 5 minutes after the VIA test in the same sitting without getting down the women from the examination table. The test sequence was conducted by the same treating doctor. Similarly, for HPV arm HPV DNA HC2 test (Hybrid Capture 2) was collected for the participating women, before and 5 minutes after VIA test in the same sitting by the same treating doctor. The test results were available usually after a week and were recorded on the same restructured and validated proforma. (Figure 1)

All women who were VIA positive, underwent Colposcopy. Directed cervical biopsies were obtained among the Colposcopy positive women. Women with confirmed cervical pre-cancers and cancers were appropriately managed.

DIAGNOSTIC TESTS

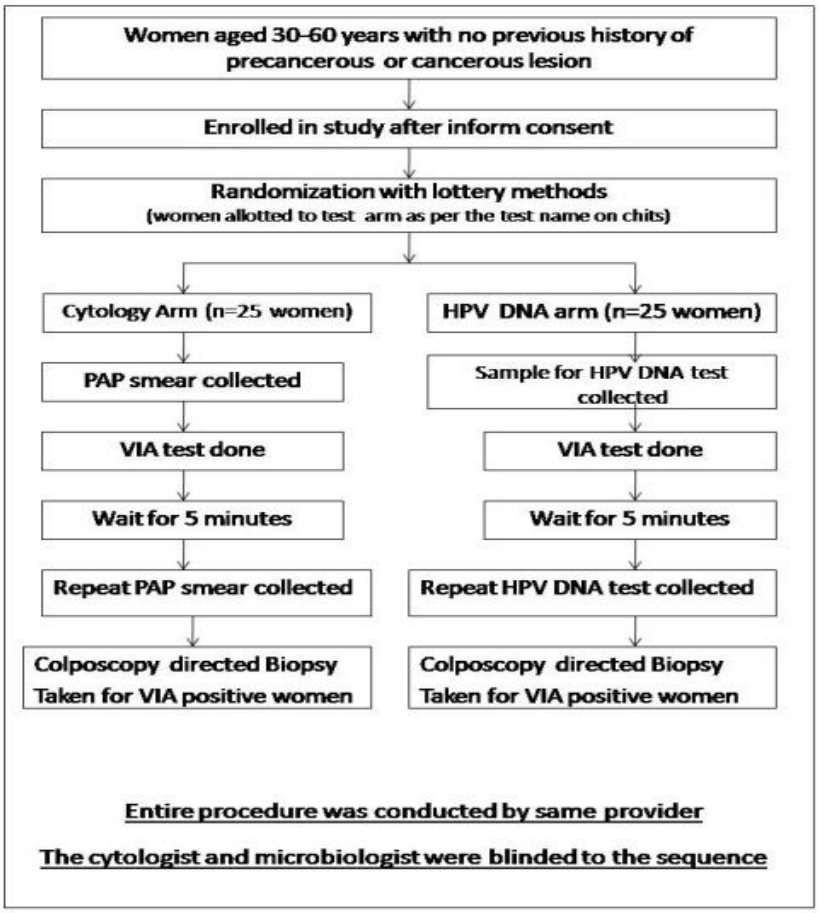

Figure 1. Work Algorithm

A. Pap smear collection and evaluation [15]

Conventional Pap smears were obtained by using sterilized, moistened cotton tipped swab stick, by standardized procedure.

The Pap smear was collected from the transformation zone, lateral vaginal wall and endocervix. The hospital has an NABL (National Accreditation Board for Testing and Calibration Laboratory) accredited Cytopathology Department, where in the smears were evaluated using the Bethesda system (recommendation 2014). The cytologist was blinded to the test sequence of Pap smear.

B. Hybrid Capture 2 Test [16]

Samples for HPV DNA test were collected using the Digene cervical sampler™ (Digene, Gaithersburg, Maryland, USA). After cleaning excess mucus with soft cotton swab the soft brush was rotated in the endocervical os in anticlock direction for three and half rounds, and then placed in the medium by breaking the tip of the sampler. The samples were coded with patient's ID code and transported to NABL accredited Microbiology department of the hospital. The Microbiologist was blinded to the test sequence of HC2 test. The signal strength of 1RLU (Relative Light Unit) or more was considered positive test.

STATISTICAL ANALYSIS

Data entry was done in the Department using SPSS version 18. Since the present study is a Pilot study percentage were calculated for agreements of results before and after VIA test. The primary objective of the study was analysed using Mc-Nemar's test in R software without continuity correction.

CYTOLOGY ARM

Pap smears were taken for 25 women before and 5 minutes after application of Acetic acid in the same sitting by the same treating doctors. The mean age of the women in this arm was 45 yrs (range 32 yrs to 58 yrs)

Table 1: Cell adequacy of Pap smears before and after VIA test.

Pap report before VIA	Pap report after VIA		Total
	Adequate	Inadequate	
Adequate	17 (71%)	7 (29%)	24
Inadequate	0	1	1
Total	17	8	25

Result Table 1- The Pap smear of one woman was reported inadequate both before & after application of acetic acid (VIA). Among the remaining 24 women, 71% women had adequate cellularity on smears both before & after VIA test, while in 29% women with adequate smears before application of VIA, were reported to have inadequate smears after application of the test. There was statistically significant difference in proportion of women reporting the cell adequacy on Pap smear before and after VIA test using acetic acid. (p=0.008)

Table 2: Colposcopy and Histopathology findings: changes in Pap smear before and after VIA.

Pap smear before VIA	Pap smear after VIA	No of pap smears	Colpo findings	Cervix biopsy
Normal	Normal	17	Normal	-
LSIL	Normal	1	Low grade lesion	CIN-I
ASCUS	ASCUS	1	Tricomonas vaginalis infection.	-
Normal	Inadequate	7	Normal	-
Inadequate	Inadequate	1	Normal	-

As seen in Table 2, the application of acetic acid (VIA test) did not alter interpretation of ASCUS smear, while a smear reported as LSIL before VIA test was reported as normal after VIA test. Colposcopy & Histopathology confirmed the findings as CIN I. (cervical intraepithelial neoplasm grade 1)

HPVDNA ARM

HPV samples were collected for 25 women by Hybrid Capture 2 method before and 5 minutes after application of acetic

acid. Mean age of women in this arm was 47 yrs (range 30 yrs to 60 yrs)

Table 3: Results of HPVDNA HC2 before and after application of VIA

HPVDNA Test before VIA	HPVDNA Test after VIA		Total
	Positive	Negative	
Positive	2	0	2
Negative	1	22	23
Total	3	22	25

As seen in Table 3, concordance was seen in results of 24 women (2 positive result and 22 negative results) before & after application of acetic acid (VIA). Only one woman whose test was negative before VIA was reported in grey zone after VIA test. (RLU of 0.8 to 1.2) An overall agreement of 96% was observed in HC2 results. There was no statistically significant difference in proportion of women with HPVDNA test results before and after application of acetic acid. (p=0.317)

DISCUSSION

Currently the cytology and HPV DNA based screening programs are not feasible and cost effective in LMIC due to the cost involved in infrastructure, the waiting period involved for the test results, and the need of recalling women leading to huge loss to follow up.

WHO (World Health Organization) has recommended VIA as a primary cervical cancer screening test for women in low income countries. The issue with VIA as a primary screening test is its low specificity and low positive predictive value. Some studies have tried to assess the effective cervical cancer screening strategy using VIA as primary screening test followed by a triage test with a high specificity tests, but not in the same sitting. The VIA screen positive women were asked to wait for couple of hours before the next triage test was conducted for them or were recalled next day or referred to tertiary care Centre. [17-19]

The aim of our study was to look at the feasibility of triaging VIA (test with a good sensitivity) screen positive women with either Pap test or a HPVDNA test (test with high specificity) at community level in the same sitting at a short interval of 5 minutes when women is still on examination table. This strategy would increase the programmatic yield and reduce the unnecessary referral. Only the women with screen positive results with VIA as primary screening test and positive either on Pap or HPV DNA as triage tests would be referred for further investigations at secondary or tertiary care levels. This strategy will have few referrals making it more cost effective.

In order for this strategy to succeed, the test results of the triage test should not be affected by 5% Acetic acid which is used for VIA screening procedures.

In the present study, the acetic acid had significantly affected the cellularity of Pap test while HPVDNA test results remained unaffected by acetic Acid. The other study that looked at the concordance of HPV DNA HC 2 results of women before and after VIA by Basu P. et al, [20] concluded that collection of samples for HC2 test is feasible immediately after VIA. The author reported an almost perfect agreement in HPV HC2 results (kappa 0.85) and RLU/Cut off ratios (correlation coefficient 0.92) observed between samples collected before and after VIA. The sensitivity and specificity to detect CIN2+ lesions remained unaltered even when cervical samples were collected after VIA. The author confirmed that acetic acid wash did not alter HC2 performance.

Few studies have evaluated the influence of acetic acid on cytology reports and it is assumed that application of acetic acid may alter the results, if the test was done immediately after VIA test as cellularity is assumed to be affected by acetic acid.

In the present study the cellularity of cytology smears was affected in 29% of women which were inadequate for reporting after application of acetic acid and there is possibility of results being altered in dysplastic smears also.

Hoellen F et al [21] studied the influence of acetic acid on pap smear of

dysplastic lesion and observed alteration of the results of the Pap smear after acetic acid application in 41% cases. However, these alterations did not result in dysplastic cases being classified as a normal smear or vice versa.

Similarly, Hornemann A et al [22] studied the influence of acetic acid on the Pap smear and concluded that acetic acid test does not seem to alter the results of the non-dysplastic smear while the dysplastic smear seems to be influenced by the acetic acid.

Performing a Pap smear before the colposcopic evaluation sometimes leads to petechial hemorrhages in older women due to atrophic changes of cervix or bleeding from ectropion in reproductive age women making Colposcopic evaluation difficult. A study that looked at a strategy of performing a Pap smear test at the end of the colposcopic examination concluded that the acetic acid used during the procedure resulted in poor cytologic evaluation. [23]

CONCLUSION

In any population-based screening program, the compliance to follow up for diagnostic workup always remains a challenge, especially in developing countries. In this process of referring there is huge loss to follow up, as the women are asymptomatic and does not understand the need of further diagnostic referral. To reduce the referrals and to reduce the attrition during referrals, a secondary triage immediately with a more specific test is required.

Screening programmes using VIA as primary screening test which has a good sensitivity and triaging the screen positive women with HPVDNA test which has a good specificity in same sittings appears to be a feasible and effective screening strategy at community level. While triaging a VIA screen positive women with pap smears in same sitting is likely to influence the results and cellularity of the smear with high potential to affect the results of a dysplastic smear. This will compromise the test characteristic of the Pap.

From this study HPV HC2 test appears promising as a immediate triage test among VIA positive women. But this needs confirmation with a larger sample size study. These findings are extremely crucial in the scenario that National Cancer Control Programme in many low-income countries including India are adopting VIA based screening and there is unavailability of enough resources to handle the large number of false positives emerging from positive VIA tests. With availability of simple and reliable HPV DNA test in low and middle-income countries and Care HPV and GeneXpert at horizon to deliver results within few hours at field level, the results of triage test could also be obtained in the same sitting. The findings have extra relevance in terms of screen and treat strategies wherein overtreatment can be minimized.

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