Concordance Between Hybrid Capture 2 Results Performed on Cervical Samples Obtained Before and Immediately After Visual Inspection with Acetic Acid Test

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ABSTRACT

Background: VIA is a simple, inexpensive test widely advocated for resource-limited settings. Major limitation of VIA is its low specificity. HPV DNA testing can be used to triage VIA-positive women if the facilities are available. The major concern for such strategy would be whether sample collection after acetic acid wash will alter HPV test characteristics. This study aimed to evaluate whether samples for HPV testing by Hybrid Capture 2 (HC2) technology can be collected immediately after VIA without altering test performance.

Methods: Total 204 VIA-positive women were recruited. Cervical samples were collected for HC2 test before and after VIA at the same sitting by the same provider. The paired samples were analyzed at the same laboratory by the same technician in the same batch of testing. Agreement in HC2 results between pre-VIA and post-VIA samples was estimated using kappa statistics. All women had colposcopy and biopsies were obtained if colposcopy was suspicious of neoplasia. Sensitivity and specificity of HC2 test in detecting CIN2+ lesions were calculated using negative colposcopy or biopsy as the gold standard and were compared between the pre and post VIA samples.

Results: Almost perfect agreement in HC2 results (kappa=0.85) and RLU/Cut off ratios (correlation coefficient=0.92) was 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. This confirmed that acetic acid wash did not alter HC2 performance.

Conclusions: Collection of samples for HC2 test is feasible immediately after VIA.

Keywords: Cervical cancer screening, hybrid capture 2, triaging, visual inspection with acetic acid, viral load

Original Article

INTRODUCTION

Visual inspection after application of acetic acid (VIA) is an inexpensive cervical cancer screening test, which can be performed even in low resource settings by trained non-clinician

providers. VIA sensitivity has been evaluated by a number of cross-sectional studies and has been observed to be significantly better than that of conventional cytology performed in the same setting.[1,2] A major advantage of the test is that the results are immediately available, which allows further management decisions to be made at the same visit. However, the positive predictive value of VIA is suboptimal, leading to unnecessary referrals and/or treatment.[3,4] A triaging strategy, if found effective, will reduce such needless referrals and/or treatment.

Hybrid Capture 2 (HC2, Qiagen INC, Gaithersburg, USA) test to detect high-risk human papillomavirus (HPV) is highly sensitive and reasonably specific when used to screen women aged 30 years or above.[5] HPV testing has been shown to have better performance than either cytology or VIA for primary screening due to much higher test sensitivity even in low and medium resource settings.[6] The test is still expensive to be considered as a primary screening test in low or medium resource settings where VIA is advocated. At some of these settings HC2 facilities can be organized, since the test is simple and requires less sophisticated laboratory infrastructure. Where feasible, the HC2 test can be used to triage the VIA-positive women for subsequent evaluation with colposcopy and/or treatment. Such a strategy will significantly reduce unnecessary referrals and will require the expensive HC2 test to be performed only on select cases. This has further relevance since a lower-cost, easier-to-use HPV test (CareHPV™), designed for low-resource settings is now available.[7]

If this new paradigm of screening is practiced, the samples for HC2 test should be taken immediately after VIA to spare the women of additional visits. There is a concern that the HC2 test results may get altered if the samples are collected after acetic acid wash. Till date, no study has been performed to address this critical issue, based on which the strategy of 'primary screening by VIA followed by triaging with HC2' can be considered. The present cross-sectional study aimed to evaluate the following:

- Agreement between the results of HC2 when cervical samples for the test were obtained immediately before and immediately after VIA test from the same women
- Correlation between RLU/cutoff ratio of HC2 when cervical samples were obtained immediately before and immediately after VIA test
- Alteration of sensitivity of HC2 to detect CIN2+ lesions if the cervical samples were collected after acetic acid wash.

METHODS

In a community-based study conducted by Chittaranjan National Cancer Institute (CNCI), India, non-pregnant women between 30 and 60 years of age with intact uterus and no previous history of cervical neoplasias were screened for cervical cancer both by HC2 and VIA. At the time of screening, the trained health workers first collected cervical samples for HC2 using the cervical brushes included in the collection kits supplied by Qiagen. The health workers then performed VIA by applying 5% acetic acid on the cervix with cotton swabs for 1 minute. If there was a dense aceto-white area with distinct margin and abutting on the squmo-columnar junction of the cervix, the woman was considered to be VIA positive as per the VIA manual published by International Agency for Research on Cancer (IARC), Lyon.[8] For the current study, a second sample for HC2 was obtained using a fresh collecting brush by the same health worker from 204 consecutive VIA-positive women immediately after performing VIA. Both the samples (pre-VIA and post-VIA) were sent on the same day to the laboratory at Chittaranjan National Cancer Institute for HC2 test. HC2 testing was performed using the high-risk probe set, which detects 13 carcinogenic HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Signal strengths in relative light units (RLU) were compared with 1 pg/mL HPV type-16 DNA positive controls (PC), and specimens with RLU/PC ratios of one or greater were judged positive.[9] The paired samples obtained from the same women were processed and tested at the same sitting in the same batch by the same technician.

All the women had colposcopy by trained clinicians after the second sample was collected for HC2. Punch biopsies were obtained if colposcopy was abnormal. The biopsies were evaluated using the CIN system of classification by a CNCI pathologist.

The degree of agreement between each pair of observations (pre- and post-VIA HC2 test

results from the same woman) was calculated by kappa statistics. It is generally accepted that a kappa value between 0.81 and 1.0 reflects almost perfect agreement, between 0.61 and 0.80 suggests substantial agreement, between 0.41 and 0.6 indicates moderate agreement, and a value <0.4 represents poor agreement. To measure the strength of associations between the paired quantitative variables (RLU/PC ratios of pre- and post-VIA samples from same woman), we calculated the correlation coefficient (*r*). A correlation coefficient of +1 indicates perfect positive correlation. Since all the women had colposcopy and biopsy (if colposcopy was abnormal), the test characteristics could be directly estimated using sensitivity, specificity, and predictive values without verification bias. To calculate test performance of HC2 to detect CIN2+ lesions, histology diagnosis or, in absence of histology, the diagnosis of normal colposcopy was considered as gold standard. Such estimation was made using both the pre-VIA and post-VIA HC2 results and were compared.

The study was approved by the Research Ethics Committee of the Institute and all the women provided written informed consent before participating in the study.

RESULTS

The study included 204 VIA-positive women who underwent HC2 test before and immediately after VIA examination. The mean age of the study participants was 35.8 ± 6.1 years. A total of 198 women had concordant HC2 result between pre- and post-VIA samples [Table 1]. The overall observed agreement between the pre- and post-VIA HC2 test results was 97.0%. The Kappa value was 0.85 (95% CI 0.73-0.96), indicating almost perfect agreement between the pre- and post-VIA HC2 test results.

Figure 1 illustrates the correlation between the viral load (RLU/PC ratio) of pre-VIA and post-VIA cervical samples. The result shows excellent correlation in viral load between the paired samples (r = 0.92).

Among the 204 VIA-positive study participants, a total of 13 women were detected to have biopsy-proved CIN2 or worse lesions, which included 4 CIN2, 7 CIN3, and 2 invasive squamous cell carcinomas. The positive predictive value of VIA was only 6.4%

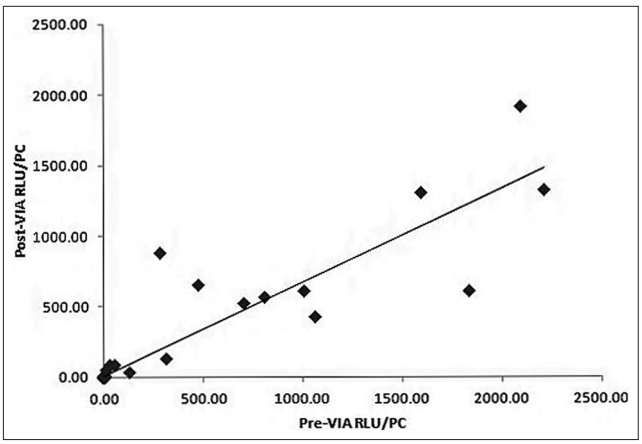

Figure 1: Scatter plot showing correlation between the viral loads estimated by HC2 test of the pre-VIA and post-VIA samples obtained from the same subjects

Table 1: Results of HC2 test from samples collected before and after VIA from 204 women (concordant samples are indicated in bold)

	Post-VIA HC2 results		
	Negative	**Positive**	**Total**
Pre-VIA HC2 results			
Negative	179	3	182
Positive	3	19	22
Total	182	22	204

HC2=Hybrid capture 2, VIA=Visual inspection after application of acetic acid, Kappa=0.85 (95% CI 0.73-0.96)

for CIN2+ diagnosis. The HC2 positivity by histology diagnosis is shown in Table 2. The sensitivity and specificity of HC2 test to detect CIN2 or worse lesions (84.6% and 94.2%, respectively) were same for both pre-VIA and post-VIA samples, since the test results did not alter for the CIN2+ lesions after acetic acid wash. The positive predictive value of HC2 in the VIA-positive women to detect CIN2 or worse lesion was 50%, which was also not affected by application of acetic acid.

The details of the 6 women with discordant HC2 results between the paired samples are shown in Table 3. The viral loads of all the discordant samples except one were close to the RLU/PC cutoff threshold of 1. None of the women with discordant HC2 result had CIN 2 or worse lesion.

DISCUSSION

The present study clearly shows that obtaining samples for HC2 test after acetic acid wash of the

Table 2: The HC2 test positivity in the pre-VIA and post-VIA samples among subjects with no neoplasia and those with different grades of neoplasias

Histology diagnosis	HC2 positivity (%)	
	Pre-VIA	Post-VIA
Normal (*N*=141)	8 (5.6)	7 (5.0)
CIN 1 (*N*=38)	3 (7.9)	3 (7.9)
CIN 2 (*N*=4)	2 (50.0)	2 (50.0)
CIN 3 (*N*=7)	7 (100.0)	7 (100.0)
Invasive cancer (*N*=2)	2 (100.0)	2 (100.0)
Colpo normal; biopsy not done (*N*=12)	0	0

CIN=Cervical intra-epithelial neoplasia, HC2=Hybrid capture 2, VIA=Visual inspection after application of acetic acid

Table 3: The HC2 test RLU/PC cutoff values among the women who had discordant HC2 test results in the pre-VIA and post-VIA samples

Test results	No. of women	RLU/PC cutoff values		Histology diagnosis
		Pre-VIA	Post-VIA	
Pre-VIA: HC2 –ve;	3	0.11	1.07	Chronic cervicitis
Post-VIA:		0.24	1.05	Normal
HC2 +ve		0.52	1.46	CIN 1
Pre-VIA:	3	1.24	0.22	HPV changes
HC2 +ve;		1.43	0.06	HPV changes
Post-VIA:		3.30	0.61	CIN 1
HC2 –ve				

CIN=Cervical intra-epithelial neoplasia, HC2=Hybrid Capture 2, HPV=Human papillomavirus, PC=Positive controls, RLU=Relative light unit, VIA=Visual inspection after application of acetic acid

cervix for 1 minute does not significantly alter either the HC2 test result or the estimated value of the viral load. More importantly, collection of sample after VIA has no impact on the test performance to detect the CIN2+ lesions. The observations are quite significant if we consider a screening paradigm in which VIA will be used as the primary screening test and HC2 will be used to triage the VIA-positive women.

Physiological changes such as immature metaplasia, reparative changes after inflammation and subclinical HPV infection are the common reasons false-positive VIA. Sending all the VIA-positive women for colposcopy will not only increase the burden on the colposcopy clinics but will also offset the cost-saving expected out of using an inexpensive test. To avoid unnecessary referrals, the test provider can collect sample for HC2 as soon as he/she makes a diagnosis of positive VIA, since there is no impact on the test results even if the samples are collected after VIA. In the present study, only 10.8% of the VIA-positive women were positive on HC2. Our overall VIA positivity was 7.0%, which implies that less than 1% women would require colposcopy referral based on HC2 triaging results. HC2 is a robust, simple test, which requires less sophisticated laboratory equipment and personnel than the Pap test. The additional benefit will be that because of the high negative predictive value of HC2, the VIA-positive but HC2-negative women will require less frequent follow-ups. A strategy of 'screen and treat' has been proved to reduce the burden of high-grade cervical cancer precursors and the effect is much more pronounced if HC2 test is used to screen.[10] VIA followed by triaging with HC2 for cryotherapy will significantly reduce the number of over-treatment in such a scenario.

Some of the studies have looked into alternate triaging options for VIA-positive women. These are VIA with magnification (VIAM), visual inspection with Lugol's iodine (VILI) and conventional cytology.[11,12] It is already established by several studies that magnification does not improve the VIA results and has no additional advantage.[13] VILI as a screening test also suffers from the problems of high false-positive results and is unlikely to provide any additional benefit as a triaging option. The study evaluating cytology to triage VIA-positive women observed poor sensitivity of such an approach.[12] The authors of the study concluded that it is better to refer all VIA-positive women for colposcopy in spite of the fact that such an approach will lead to many unnecessary biopsies. Moreover, cytology cannot be obtained once the cervix is washed with acetic acid and the woman will require an additional visit.

Previous studies evaluated the concordance of HC2 results when same samples were analyzed in different laboratories and observed high inter-observer agreement.[6] The present study throws light on the intralaboratory variation and again establishes the robustness of HC2 test.

Considering all the options, HPV DNA detection by HC2 test (or CareHPV when available) seems to be the best one for triaging VIA-positive women.

However, any new strategy has to be appropriately evaluated before being recommended in clinical practice. The same is applicable for the role of HC2 in triaging VIA-positive women and a large population-based study is currently ongoing at our institute. In the present publication, we establish only the feasibility of such a strategy, since collection of samples after VIA does not affect HC2 test results.

CONCLUSIONS

Cervical samples for HPV detection by HC2 test can be collected after VIA test since no change in the HC2 test characteristics were observed in our study even if samples were collected after acetic acid wash.

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