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Evaluation of Visual Inspection by Acetic Acid, High Risk Human Papilloma Virus Testing and Human Papilloma Virus 16/18 Genotyping to Screen for Cervical Cancer in a Low Resource Setting

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Abstract: ***Cancer Cervix screening has evolved over the years and newer modalities for cancer cervix are constantly emerging. This study was undertaken to analyse the sensitivity and specificity of VIA, HR HPV testing and HR HPV testing triaged by HPV 16/18 genotyping with Colposcopy and Biopsy as gold standard. Out of 402, 51 women (12.7%) were positive on one or more screening test and 12 women were identified with Cervical Intraepithelial Neoplasia (CIN 1 or worse). The sensitivity of Pap Smear, VIA, HR HPV, HPV triage and sequential testing was 58.3%, 91.7%, 88.3%, 90% and 75% respectively and the specificity was 86.4%,61.7%,79%,88.3% and 88.9% respectively. Thus, the best balance of sensitivity and specificity was of HR HPV triage with genotyping and Sequential testing. In countries where it is feasible, HR HPV testing as a primary test and triage with HPV 16/18 genotyping or VIA or cytology can be undertaken and in resource poor countries primary screening by VIA and sequential testing for HR HPV can be done for screening.***

Keywords: Visual Inspection by Acetic Acid, Pap Smear, HPV testing, Cervical Intraepithelial Neoplasia

1. Introduction

Cervical Cancer is the fourth most common cancer affecting women worldwide. More than one fifth of all new cases are diagnosed in India. There are 1,22,844 new cases and 67,477 deaths every year in India.[1] Although population-based screening has resulted in a substantial reduction in cervical cancer burden in developed countries, lack of screening or inefficient screening programs contribute to high risk seen in sub -Saharan Africa, South and Southeast Asia, Oceania, central and south America, and the Caribbean.[2,3]

As the process from developing precancerous lesions of the cervix after persistent infection with Human Papilloma Virus to becoming invasive cervical cancer often takes 10 to 15 years to develop, it provides many opportunities for detection and treatment of precancer lesion.[10] These are asymptomatic lesions which can be easily detected by use of effective screening methods. If detected early, there is almost 100% cure rate with simple procedures, while advanced cancers have less than 35% survival rates.[4]

The problem is that in developing countries like India, universal screening has not been achieved. Cytology based screening programmes are difficult to organize in India owing to limited infrastructure, trained personnel, and funds. Thus, in developing countries like ours, there is a need for alternative screening strategies which are available on a large scale, easy to perform and can be done in peripheral areas.

Thus, this present study was undertaken to evaluate alternative screening strategies and triage method for detection of premalignant and malignant lesions of the cervix.

Aims and Objectives

1) To analyse the sensitivity and specificity of VIA, HR HPV testing and HR HPV testing triaged by HPV 16/18 genotyping with Colposcopy and Colposcopy Directed Biopsy as gold standard.
2) To assess the sensitivity and specificity of sequential testing of both VIA followed by HPV and HPV followed by VIA.

2. Materials and Methods

This study was carried out in the Department of Obstetrics and Gynaecology, Maulana Azad Medical College and associated Lok Nayak Hospital for a duration of two years.

Study Design: Cross sectional Prospective study

Study Population: 402 women in the age group 30-65 years of age attending the Gynaecology OPD or admitted in the gynaecology ward at Lok Nayak Hospital were included in the study.

Inclusion Criteria: Women in the age group 30-65 years presenting to the gynaecology OPD or admitted in the gynaecology ward.

Exclusion Criteria: The following women will be excluded from the study-

1) Pregnant women
2) Women with active bleeding per vaginum
3) Known cases of carcinoma endometrium
4) Known cases of carcinoma cervix
5) Women with a frank growth on cervix
6) Women who have been previously treated for CIN or carcinoma cervix

7) Women with history of pelvic irradiation

3. Methodology

402 women in the age group 30-65 years which presented to the gynaecological clinic of Lok Nayak Hospital for a duration of two years were recruited in the study. Written informed consent was taken after explaining the procedure to the women. At first visit detailed history, general examination was done and then theywere subjected to per speculum examination and samples were taken for cervical cytology for-

a) **Pap smear** which was done using conventional cytology, the cervical scrapes were taken from ectocervix and endocervix using an Ayre's spatula and an endocervical brush. The material was smeared on a pre-labelled slide and was immediately fixed by dipping the slide in 95% ethyl alcohol. The pap smears were processed and graded according to the modified Bethesda system. Pap Smear was considered positive in reported cases of ASCUS and above.
b) **High risk HPV DNA**(HR HPV) testing which was done by DigeneHybrid Capture II (HC II) which is an in vitro microplate assay based on signal-amplified nucleic acid hybridization that used chemiluminescence for the qualitative detection of 18 types of HPV DNA in cervical specimens. Samples were collected (before application of acetic acid) with the help of cytobrush and transported in Sample Transport medium (STM). Assay results are reported as Reactive Light Units (RLU) of HPV DNA in the sample. A sample is considered positive if the ratio of the sample RLU to the positive controls is ≥ 1.0.

Visual Inspection by Acetic Acid
After sending the above samples per vaginal examination was done and then **Visual Inspection by Acetic Acid** was done in all patients which was done by smearing the cervix with a cotton swab dabbed in 5% acetic acid solution and the findings were reported after one minute. A distinct acetowhite area within the transformation zone was considered VIA positive.

HPV 16/18 Genotyping
Further **HPV 16/18 genotyping** was done if the women was positive for HPV DNA tested by HC II. The Genotyping was done by HPV DNA Polymerase Chain Reaction (PCR) for the detection of HPV 16 and 18 which are known to cause 90% of cervical premalignant and malignant lesions.

Sequential Testing
Combining two tests for screening of cervical cancer improves the overall performance and reduces the colposcopy referral rate. We studied sequential testing by combining VIA and HR HPV DNA testing. In one combination we did VIA in all screening population and those who tested positive on VIA were referred for HR HPV DNA testing.

In the other combination all of the screening population underwent HR HPV DNA testing by HC II and those who tested positive were further sequentially tested by VIA and in both combination the cases which were positive by both the test were referred for Colposcopy and Biopsy.

Colposcopy
Colposcopy was done for women who tested positive for any of the screen test. Colposcopy was performed using a video colposcope (Digital Colposcope with workstation, Goldway). The cervix was inspected in good light. Mucus or any vaginal discharge was removed with saline and any area suggestive of leucoplakia was noted. Green filter was used to look for abnormal vessels. Then 5% freshly prepared acetic acid solution was liberally applied over the cervix and vaginal walls using a cotton tipped applicator. After one minute of acetic acid application the entire cervix was closely examined under magnification ranging from 5-25 X. The cervix was then examined after application of Lugol's iodine solution so that any abnormal areas of iodine non-uptake could stand out as mustard/canary yellow against the mahogany brown colour of the normal squamous epithelium.

Biopsy-
Cervical biopsies were taken from the abnormal areas noted on the colposcope in the women referred for colposcopy and if the colposcopy was normal random biopsy was taken if the women tested positive from any of the screening test. Biopsy were taken with a punch biopsy forceps or a loop biopsy was taken using electrosurgical unit, or a cone biopsy was done if indicated. The specimen was fixed in 10% formalin and sent for processing.

Several women in the study group who were screen negative underwent hysterectomy due to other gynaecological problems (fibroid uterus, prolapsed, ovarian cyst, etc). These were taken as controls.

Biopsies revealing CIN 1 or worse lesions were considered positive and considered as true positive cases.

Statistical Analysis

Data was entered in excel and analysed using SSPS version 17.

The sensitivity, specificity, positive predictive value, negative predictive value and accuracy of various screening tests were calculated with histopathology as a gold standard.

4. Observation and Results

402 women in the age group 30-65 years were screened with Pap smear, VIA, HPV DNA testing by Hybrid Capture II and those positive by HC II were sequentially tested by HPV 16/18 genotyping by DNA PCR. Those found positive on any or all the screening were subjected to colposcopy and cervical biopsy. Biopsies were done for 51 screen positive women and 42 screen negative women which were taken as controls. The results were compiled and analysed.

Table 1: Sociodemographic Profile of the Screening Population

Characteristics	Number of women (n=402)
Age (years)	
Mean ± SD	42.76±7.63 years
Median (range)	42 years (30-65)
Parity	
0-3	59.7%
>3	40.3%
Socioeconomic status (modified Kuppuswamy scale)	
Lower	74.4%
Middle	25.1%
Upper	0.5%
Education	
No schooling	48%
Primary School	8.5%
High School	28.4%
>High School	15.1%
History of smoking	7.2%

The presenting complaint was vaginal discharge in 35% of the women, 14.6% had irregular bleeding per vaginum, 2.9% women complained of postcoital bleeding.

Pap Smear was called positive in reported cases of ASCUS and above. The following were the results of various screening test in a study population of 402 women.

Table 2: Results of Various Screening Test (n=402)

Test	Positive cases	Percentage out of total population (n=402)
Pap smear	18	4.5%
VIA	42	10.4%
HPV by HC II	27	6.7%
HPV 16/18 Genotyping	11	2.7%

42 women (10.4%) were found positive on screening with VIA which were the maximum, Pimple et al[5] in her study had found a positivity rate of 9.5% which is similar to our results.

This led to total referral of 51 women for colposcopy and biopsy which had the following results.

Table 3: Result of Biopsy of Screen Positive Cases (n=51)

Biopsy	No.	% of screen positive (n=51)	% of total population (n=402)
Cervicitis	39	76.5	9.7
CIN 1	5	9.8	1.2
CIN 2	4	7.8	1.0
CIN 3	2	3.9	0.5
Invasive cancer	1	2.0	0.2
Total	51	100.0	12.7

Biopsy was considered positive in cases with report CIN 1 and above, hence 12 (23.5%) women were found positive on Biopsy. CIN 2 or worse lesions were positive in 9 cases (17.6%).

Each screening test was individually studied and its agreement with the biopsy report was studied to calculate the performance characteristics of the test, i.e., Sensitivity, Specificity, Positive and negative Predictive value, accuracy and kappa value.

To avert verification bias 42 screen negative women underwent cervical biopsies and were included in the calculation of the performance results of individual screening tests.

Table 4: Comparative Analysis of Various Screening Methods For CIN 1 or Worse

Test	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa
PAP SMEAR	58.3%	86.4%	38.9%	93.3%	83.8%	0.23
VIA	91.7%	61.7%	26.2%	98.1%	65.6%	0.26
HPVDNA (HC II)	88.3%	79%	37.1%	96.9%	79.6%	0.41
HPV 16/18 TRIAGE OF HC II POSITIVE	90%	88.3%	81.8%	93.7%	88.9%	0.76
VIA →HC II	75%	88.9%	50%	96%	87.1%	0.43
HC II→VIA	75%	88.9%	50%	96%	87.1%	0.43

- The sensitivity was highest for VIA (91.7%), followed by HPV 16/18 genotyping (90%), and was lowest for Pap smear (58.3%).
- The specificity was highest for Sequential testing (88.9%), followed by HPV 16/18 genotyping (88.3%), and was the lowest for VIA (61.7%).
- Accuracy was highest for HPV 16/18 genotyping (88.9%) and sequential testing (87.1%) and lowest for VIA (65.6%).

Since a large number of CIN 1 lesions regress it is important to see the efficacy of screening tests for high grade lesions (CIN 2 or worse).

Table 5: Comparative Analysis of Various Screening Tests for CIN 2 OR WORSE

Test	Sensitivity	Specificity	PPV	NPV	Accuracy	Kappa
Pap smear	71.4%	94.8%	27.7%	97.3%	83.8%	0.26
VIA	100%	59.3%	16.7%	100%	62.4%	0.18
HPV DNA (HCII)	85.7%	75.6%	22.2%	98.5%	76.3%	0.27
HPV 16/18 TRIAGE OF HC II	100%	76.19%	54.55%	100%	81.48%	0.35
VIA→HC II	85.7%	86.1%	33.3%	98.6%	86.1%	0.24
HC II→VIA	85.7%	86.1%	33.3%	98.6%	86.1%	0.24

- The sensitivity was highest for VIA and HPV 16/18 genotyping (100%), followed by HR HPV by HC II and sequential testing (85.7%) and was found the lowest for Pap smear (71.4%).
- The specificity was highest for Pap smear (94.8%), followed by sequential testing (86.1%) and was found the lowest for VIA (59.3%).
- Accuracy was highest for sequential testing and HPV 16/18 genotyping (86.1%), followed by Pap smear (83.8%) and was found lowest for VIA (62.4%).

5. Discussion

Cervical cancer is one of the major cause of cancer related morbidity and mortality in women in India. Various screening programmes have been implemented in our country based on Pap smear which has a poor sensitivity, we also found a poor sensitivity of 58.3% for detecting CIN 1+ lesions also it requires infrastructure for its processing, the majority of the women affected by cancer cervix belong to lower socioeconomic status, hence cytology based screening have been seen to have a low coverage rate and loss to follow up in developing nations.

This creates a need for an effective approach for screening of Cervical Cancer in low resource settings, alternative methods for screening like VIA was studied by us which showed a high positivity rate of 10.4% in our study but VIA detected 11 out of 12 CIN 1+ lesions and showed a sensitivity of 91.7% for CIN1+ and as high as 100% for CIN 2+, but the specificity of VIA was low which was 61.7% for CIN 1+ and 59.3% for CIN 2+.

The discovery of the role of HPV in the causation of Cancer Cervix and its premalignant lesions has led to the development of various methods of HPV testing, we evaluated the performance of HPV testing by HC II, which detected 10 out of 12 cases of CIN 1+ and showed a sensitivity of 88.3% for CIN 1+ as it missed two cases and 85.7% for CIN 2+. HPV testing is known to have relatively lower specificity (79% for CIN 1+ in our study) and poor positive predictive value (37%), leading to higher referral rate for colposcopy and many of these women will not be found to harbour any lesion. To improve the overall performance for detection of premalignant and malignant lesion of cervix there is a need for triaging and sequential testing. We triaged HPV testing by genotyping to detect HPV 16/18, since it has been found that 70% of the cancer cervix are associated with HPV 16 or 18 or both.[6]Triaging improved the sensitivity to 90% for detection CIN1+ and 100% to CIN 2+.

By combining two tests we can improve the specificity without much difference in sensitivity this approach was applied in the concept of sequential testing which combined two tests, i.e, VIA and HPV. The results of sequential testing showed that the specificity for detecting CIN1+ was 88.9% which was higher than the individual specificity of VIA and HPV (61.7% and 79%).

Screening tests which provide immediate results should be preferred in a country like ours because of a high loss to follow up rate, VIA is one such test which has immediate results but due to its poor specificity (61.7% for CIN 1+) and poor positive predictive value (26.2% for CIN 1+) has a very high Colposcopy referral rate, sequential testing with HPV testing has found to improve overall performance and reduced referral for Colposcopy.

6. Conclusion

In countries where it is feasible, HR HPV testing as a primary test and triage with HPV 16/18 genotyping or VIA or cytology can be undertaken and in resource poor countries primary screening by VIA and sequential testing for HR HPV can be done for screening.

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