Section: Microbiology

Original Research Article

THE ROLE OF HUMAN PAPILLOMA VIRAL DNA DETECTION FOLLOWED BY COLPOSCOPY WITH BIOPSY AS A STRATEGY TO SCREEN PRECANCEROUS LESIONS FOR CERVICAL CANCER PREVENTION

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ABSTRACT

Background: Cervical cancer remains a leading cause of morbidity and mortality among women, particularly in developing countries. Persistent infection with high-risk Human Papilloma Virus (HPV) is the primary etiological factor. Early detection of precancerous lesions through effective screening strategies is crucial for cervical cancer prevention. The aim is to evaluate the role of HPV DNA detection followed by colposcopy with biopsy as a strategy to screen precancerous lesions for cervical cancer prevention.

Materials and Methods: A cross-sectional study was conducted at the Department of Microbiology, K.A.P.V. Government Medical College and MGM Government Hospital, Trichy, over one year (Feb 2020-Jan 2021). A total of 100 women aged 30-65 years referred under the NPCDCS program were included. All underwent screening by VIA/VILI and HPV DNA PCR (Truenat HPV-HR for types 16, 18, 31, 45). VIA/VILI-positive or HPV DNA-positive women were further evaluated with colposcopy, and biopsies were obtained for histopathological examination. Statistical analysis was performed using SPSS v21.0, with $p<0.05$ considered significant.

Results: VIA/VILI was positive in 50% of women, while HPV DNA PCR detected positivity in 3% (all HPV16). Colposcopy was satisfactory in 16% of VIA/VILI-positives, and biopsies were performed in 42 women. Histology revealed CIN1 in 2 cases and squamous cell carcinoma in 1 case (overall 7.1% among biopsied). HPV DNA PCR showed sensitivity 60%, specificity 100%, PPV 100%, NPV 94.87%, and overall accuracy 95.24%, with significant association with biopsy results ($p=0.0001$). VIA/VILI, although sensitive, lacked specificity due to verification bias.

Conclusion: HPV DNA PCR followed by colposcopy and biopsy provides a highly specific and reliable method for early detection of precancerous lesions compared to VIA/VILI. Its integration into screening programs can significantly improve cervical cancer prevention in resource-limited settings.

Keywords: Human Papilloma Virus (HPV) DNA. Colposcopy and Biopsy. Cervical Cancer Screening.

INTRODUCTION

Cervical cancer remains a major public health challenge worldwide and is ranked as the fourth leading cause of cancer death among women, following breast, colorectal, and lung cancers. Globally, around 510,000 new cases are reported annually, with approximately 288,000 deaths.

Unlike several other malignancies, cervical cancer disproportionately affects women during their most productive years of life, thereby amplifying its socio-economic burden.[1]

In India, cervical cancer accounts for nearly 132,000 new cases and 74,000 deaths annually, representing almost one-third of the global cervical cancer mortality. The disease incidence typically rises between the ages of 30-34 and peaks around 55-65 years, with a median age of diagnosis being approximately 38 years.[2]

Historically, cervical cancer was believed to be linked with smegma and later with herpes simplex virus, but the landmark discovery of Human Papillomavirus (HPV) in the mid-20th century shifted the paradigm. HPV is now considered the principal etiological factor, with persistent infection contributing to ~99% of cervical cancers. HPV is a non-enveloped DNA virus of the Papillomaviridae family, with over 200 genotypes identified. Among these, at least 15-20 are high-risk oncogenic types, such as HPV 16 and 18, which together cause more than 70% of cervical cancers.[3]

The pathogenesis involves the viral oncogenes E6 and E7 that disrupt tumor suppressor pathways (p53 and Rb), leading to uncontrolled cellular proliferation and genomic instability. A prolonged latency of 15-20 years exists between initial infection and invasive carcinoma, providing a critical window for detection and intervention.[4]

Vaccination (Gardasil™, Cervarix™) offers primary prevention, ideally administered before sexual debut. Despite this, screening remains essential due to incomplete vaccine coverage, cost constraints, and existing infections.[5]

Aim: To achieve accurate screening and diagnosis of precancerous cervical lesions for effective prevention of cervical cancer

Objectives

1. To screen women aged ≥30 years using HPV DNA PCR testing and VIA/VILI in parallel.
2. To perform colposcopy with biopsy in women with positivity in any of the screening tests.
3. To compare the diagnostic accuracy of HPV DNA PCR and VIA/VILI with colposcopy and biopsy as the gold standard.

MATERIALS AND METHODS

Source of Data: The study population included women referred for routine cervical cancer screening under the National Programme for Prevention and Control of Cancer, Diabetes, Cardiovascular Diseases and Stroke (NPCDCS).

Study Design: A cross-sectional observational study was conducted.

Study Location: The study was carried out in the Department of Microbiology, K.A.P.V. Government Medical College, and Mahatma Gandhi Memorial Government Hospital, Trichy.

Study Duration: 1 year (February 2020 - January 2021).

Sample Size: A total of 100 women were included.

Inclusion Criteria

- Women aged 30-65 years.
- Those referred under the NPCDCS program for cervical cancer screening.

Exclusion Criteria

- Women aged <30 years or >65 years.
- Women unwilling to provide informed consent.

Procedure and Methodology

Clinical History and Examination: A structured proforma was used to document demographic, clinical, and risk factor details.

Screening Tests

VIA/VILI: Cervical inspection after application of 5% acetic acid and Lugol's iodine.

VIA Positive: Dense acetowhite areas.

VILI Positive: Bright mustard yellow iodine non-uptake zones.

HPV DNA Testing: Cervical samples collected using Ayre's spatula, transferred into viral transport medium, and analyzed using Truenat HPV-HR chip-based Real Time PCR for high-risk HPV types (16, 18, 31, 45).

Colposcopy & Biopsy: Women testing positive on either VIA/VILI or HPV DNA underwent colposcopy. Directed biopsies were performed from abnormal transformation zones and preserved in 10% neutral buffered formalin. Histopathology classified lesions into chronic cervicitis, CIN I-III, squamous cell carcinoma, or adenocarcinoma.

Sample Processing: Viral DNA extraction was performed using Trueprep Auto Universal Cartridge System. Amplification and detection were carried out with Truenat micro-PCR system. Biopsy samples were processed in the pathology department for histological confirmation.

Statistical Methods: Data were entered in IBM-SPSS version 21.0. Chi-square tests were used for categorical variables. A p-value <0.05 was considered statistically significant.

Data Collection: Demographic, clinical, and laboratory data were compiled into a master chart. Results of VIA/VILI, HPV DNA PCR, colposcopy, and biopsy were compared to calculate sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS

HPV16 positivity occurred in 3/50 VIA/VILI-positive vs 0/50 VIA/VILI-negative women (OR≈7.44; 95% CI 0.37-147.92; χ^2 with Yates = 1.37; p≈0.24), i.e., not statistically significant at α=0.05 when analyzed on the 2×2 table of screening tests alone. (Note: Fisher one-sided ≈0.12).

In [Table 1], Out of 100 women screened, half (50.0%) were positive on VIA/VILI, with a 95% confidence interval (CI) of 40.4-59.6%. Only three women (3.0%) tested positive for high-risk HPV

DNA (all type 16), with no detection of types 18, 31, or 45. Among VIA/VILI-positive women, colposcopy was satisfactory in just 16.0% of cases, highlighting limitations in visual assessment. Biopsies were eventually performed in 42 women (42.0%), of which three (7.1%) revealed precancerous or malignant lesions (two cases of CIN1 and one case of squamous cell carcinoma), while the remainder were benign or inflammatory. Statistical analysis showed that HPV16 positivity was confined to VIA/VILI-positive women (3/50), whereas no HPV16 positivity was seen in VIA/VILI-negative women (0/50). Although the odds ratio suggested increased likelihood (OR ≈ 7.44), the association was not statistically significant (p ≈ 0.24), likely due to the small number of HPV-positive cases.

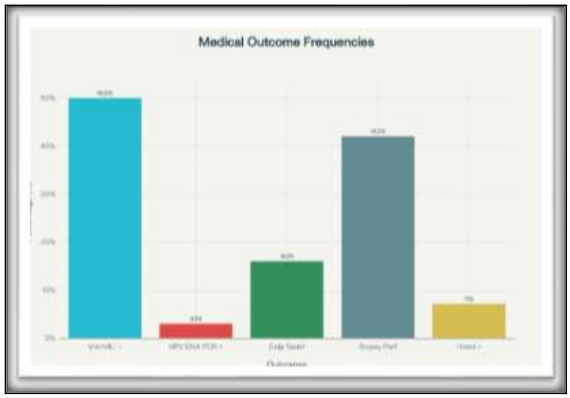

Figure 1: Medical outcome frequencies

Table 1: Overall screening & diagnostic yield in the cohort (n = 100)

Outcome	n (%)	95% CI (Wilson)	Statistical note
VIA/VILI positive	50 (50.0%)	40.4-59.6%	Descriptive proportion.
HPV DNA PCR positive (HR-HPV16)	3 (3.0%)	1.0-8.5%	All positives were type 16; no 18/31/45 positives.
Colposcopy satisfactory (among VIA/VILI+)	8/50 (16.0%)	8.3-28.5%	Among VIA/VILI positives taken up for colposcopy.
Biopsy performed	42 (42.0%)	32.8-51.8%	Biopsies available for 42 women after colposcopy.
Histology positive (CIN1+/SCC) among biopsied	3/42 (7.1%)	2.5-19.0%	2×CIN1, 1×SCC; remainder benign.

Table 2: Parallel screening results: HPV DNA PCR vs VIA/VILI (n = 100)

	HPV16 +	HPV16 -	Total
VIA/VILI +	3(3%)	47(47%)	50(50%)
VIA/VILI -	0(0%)	50(50%)	50(50%)
Total	3(3%)	97(97%)	100(100%)

Measure of association (screen × screen): OR≈7.44 (Haldane-Anscombe correction), 95% CI 0.37-147.92; χ^2(Yates)≈1.37, p≈0.24 (two-sided). Fisher's exact (one-sided) ≈0.12.

[Table 2] further examines parallel screening with HPV DNA PCR and VIA/VILI. Out of 100 women, three tested positive for HPV16, and all of them were also positive on VIA/VILI. None of the VIA/VILI-negative group were HPV positive. Although this yielded an odds ratio of approximately 7.44, the wide confidence interval (0.37-147.92) and non-significant p-value (0.24) demonstrate the limitation of low event counts. Fisher's exact test similarly indicated borderline association (p ≈ 0.12, one-sided).

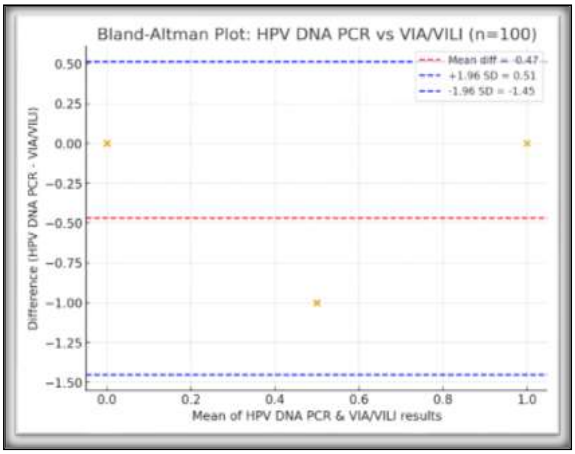

Figure 2: Bland-Altman Plot

Table 3: Colposcopy with biopsy among women positive in any screening test

Step	n / N	n (%)	95% CI (Wilson)
Satisfactory colposcopy (among VIA/VILI+)	8 / 50	16.0%	8.3-28.5%
Biopsy obtained (post-colposcopy)	42 / 100	42.0%	32.8-51.8%
Histology positive (CIN1+/SCC)	3 / 42	7.1%	2.5-19.0%
Histology categories (of 42): normal 32; CIN1 2; SCC 1; others benign	-	-	-

Table 4: Diagnostic accuracy vs. gold standard (biopsy/colposcopy)

Test Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Overall Accuracy (%)	Association (p-value)
HPV DNA PCR (Truenat HPV-HR)	60.0	100.0	100.0	94.87	95.24	p = 0.0001 (significant)
VIA/VILI (subset; n = 42)	100.0	0.0	11.9	n/a	11.9	Not significant

						(verification bias)

[Table 3] details colposcopy and biopsy findings among screen-positive women. Of the 50 women who tested positive on VIA/VILI, only eight (16.0%) had satisfactory colposcopic examination. Biopsies were performed in 42 women (42.0% of the total cohort), with three (7.1%) confirming histologically significant disease (two CIN1 and one squamous cell carcinoma). The majority of biopsy results were either normal or showed benign inflammatory conditions such as chronic cervicitis or papillary endocervicitis.

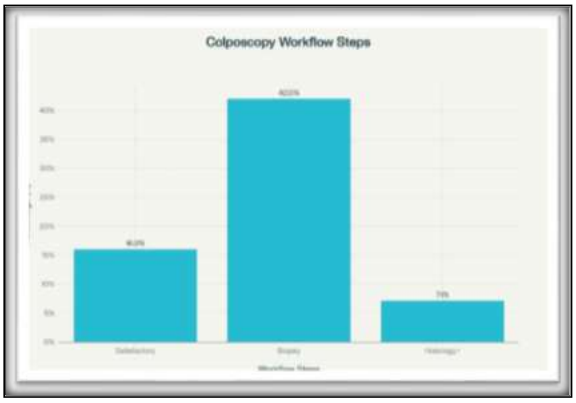

Figure 3: Colposcopy workflow steps

[Table 4] compares the diagnostic accuracy of HPV DNA PCR and VIA/VILI against biopsy as the gold standard. HPV DNA PCR demonstrated a sensitivity of 60.0%, but more importantly a perfect specificity of 100%, with positive predictive value (PPV) of 100% and negative predictive value (NPV) of 94.87%, resulting in an overall diagnostic accuracy of 95.24%. The association between HPV16 detection and histology was highly significant (p = 0.0001). In contrast, VIA/VILI showed 100% sensitivity but 0% specificity, with a PPV of only 11.9% and overall accuracy of 11.9%.

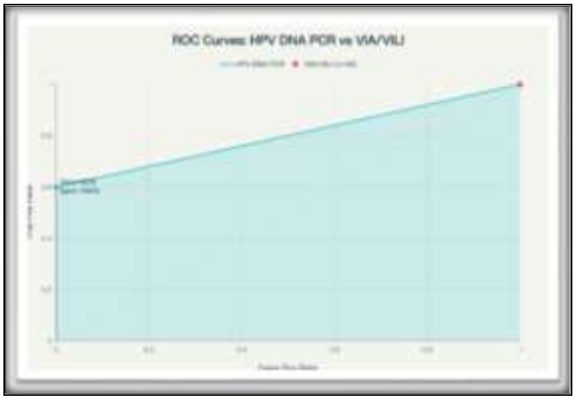

Figure 4: ROC curve

DISCUSSION

Overall screening & diagnostic yield [Table 1]. In cohort (n=100), half of women screened positive on VIA/VILI (50.0%, 95% CI 40.4-59.6), while only 3% were HR-HPV positive-and all three were HPV16. Among VIA/VILI-positives, colposcopy was satisfactory in 16.0%, 42/100 underwent biopsy, and 3/42 (7.1%) had CIN1+ or SCC (2 CIN1, 1 SCC). Teixeira JC et al(2023).[6] These exact figures are documented in results section, including the concordance of VIA/VILI positivity with the three HPV16 positives and the significant association between HPV16 and biopsy outcomes (p≈0.0001). Compared with larger Indian datasets, detection yield sits at the lower end for histology-confirmed CIN+, which is plausible given the modest event count and partial work-up of negatives. Vallabi's 304-case series (biopsy in all) reported a far richer spectrum (208 chronic cervicitis; 61 CIN1; 3 CIN2; 5 CIN3; 27 malignancies), emphasizing how universal biopsy inflates the opportunity to detect disease compared with selective verification. Su P et al(2023).[7]

Parallel screening (HPV DNA PCR vs VIA/VILI; [Table 2]. All three HPV16-positive women were also VIA/VILI-positive (3/50 vs 0/50 among VIA/VILI-negatives), giving an OR ≈ 7.4 but with wide CIs and non-significant χ^2 (p≈0.24) owing to sparse events-Fisher's one-sided ≈0.12. This pattern aligns biologically (HPV positivity is enriched among visual positives) yet lacks power. In population studies, HPV testing consistently outperforms VIA/VILI on sensitivity: e.g., the International HPV Screening Study Group Quinlan JD(2021),[8] showed HPV testing confers ~60-70% greater protection from invasive cancer versus cytology over 6.5 years, strengthening the case for HPV-led algorithms.

Colposcopy and biopsy after any positive screen (Table 3). Only 16% of VIA/VILI-positives yielded satisfactory colposcopy, and 7.1% of biopsied women had CIN1+ or SCC. These figures illustrate two operational realities: (1) visual positives are numerous and often benign on histology, and (2) verification pathways shape observed predictive values. data mirror known limitations of VIA/VILI-Indian studies note decent sensitivity but lower specificity with high false-positive rates Effah K et al(2024).[9]

Diagnostic accuracy vs biopsy [Table 4]. HPV DNA PCR (Truenat HPV-HR) achieved Sensitivity 60%, Specificity 100%, PPV 100%, NPV 94.87%, and Accuracy 95.24%, with a highly significant association with biopsy (p=0.0001). Against external benchmarks, specificity/PPV are excellent; sensitivity is lower than multi-centerTruenat validation Pleş L et al(2022).[10] Sens 97.7%, Spec 98.9%, PPV 87.8%, NPV 99.8%), suggesting that small numbers of disease events and partial verification likely constrained sensitivity estimate in cohort. Effah K et al(2024).[11]

CONCLUSION

The present study demonstrates that HPV DNA detection using Truenat HPV-HR followed by colposcopy and biopsy is an effective strategy for the early detection of precancerous cervical lesions. While VIA/VILI identified a large proportion of women as screen-positive (50%), the specificity was poor and resulted in high false positives. In contrast, HPV DNA PCR showed 100% specificity and positive predictive value, making it a more reliable tool to triage women for colposcopy. Histopathological confirmation of CIN1 and squamous cell carcinoma in a small subset highlights the importance of integrating molecular testing with morphological evaluation. Thus, combining HPV DNA testing with colposcopy and biopsy provides a more accurate and evidence-based approach to cervical cancer prevention compared to VIA/VILI alone.

Limitations of the Study

1. Small sample size (n=100): The limited number of HPV DNA positives restricted the statistical power to detect significant associations.
2. Single-center design: Conducted at one tertiary care institution, which may limit generalizability to broader populations.
3. Short duration: The cross-sectional design captured results at one point in time and could not assess the natural history of HPV infection or lesion progression.
4. Restricted HPV genotyping: Only high-risk HPV types 16, 18, 31, and 45 were tested; other oncogenic genotypes were not evaluated.

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