The Journal of Obstetrics and Gynecology of India (January–February 2022) 72(1):13–18
https://doi.org/10.1007/s13224-021-01569-9

MINI REVIEW ARTICLE

Advances in HPV Screening Tests for Cervical Cancer—A Review

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Received: 16 July 2021 / Accepted: 8 September 2021 / Published online: 13 October 2021

Abstract
HPV is responsible for almost all cases of cervical cancer which in turn is one of the common causes of death among female genital malignancies. Cervical cancer being a preventable disease, screening plays a vital role in its reduction. In this era of advanced health care system and technologies this cancer is still in the increasing trend, especially in the Low and Middle Income Countries, which reflects the poor coverage of women for screening. Advances in screening tests and techniques for better and larger coverage of women is the need of the hour globally. Clinicians also need to be aware of the various promising technologies available for screening of cervical cancer precursors, application of which in general practice can be of immense help in cervical cancer reduction.

Keywords HPV · Cervical cancer · Screening · Tests · Techniques · Advances

Introduction

Globally, cervical cancer is ranked as the fourth most common cancer in women and the most common cancer among women with HIV [1]. Cervical cancer is preventable but is on the rise causing concern and is thus the target for reduction by WHO (World Health Organization). High-risk Huma Papilloma Virus (hr-HPV) is responsible for almost all cervical cancers. According to the International Agency for Research on Cancer, there are 14 types of hr-HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68), which are responsible for the pre-cancerous and the cancerous lesions. It has been noted that every year more than 85% of the 311 000 deaths due to cervical cancer, occur in low and middle-income countries (LMICs). [1] This is mainly attributable to the low screening rates in these areas which may, in turn, be due to the lack of awareness, motivation and facilities. WHO currently (2021) recommends 3 screening tests for HPV: 1) Nucleic acid amplification tests (NAAT) for hr-HPV types (hr-HPV DNA/NAAT and mRNA), 2) Visual inspection with acetic acid or with Lugol's iodine (VIA/VILI) by naked eye or magnified by colposcope or camera and 3) Cytology (Conventional Pap/Liquid-based cytology/Dual staining to identify p16 and Ki-67). [2] However, with the development of newer screening tests and techniques with higher sensitivities and specificities, the incidence of cervical cancer may be reduced significantly and WHO may achieve its target of 2030.

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With the volley of newer and efficient tests and techniques introduced to improve screening, it is necessary for clinicians to stay in touch with the updates, as these are introduced to cater to the needs of women at low-income settings.

Discussion

About 90% of HPV infections usually clear up within 2 years spontaneously while a small proportion of the infection with high-risk viruses can persist leading to pre-cancerous and cancerous lesions. Factors such as age, sexual behavior, immune status, HPV types, and initial treatment will affect viral clearance, however, the majority tend to clear the virus within 6 to 12 months of infection. [3, 4] It takes 15 to 20 years in women with normal immunity to develop cervical cancer while 5 to 10 years in immunocompromised women (women living with HIV). HPV is now known to be the most common sexually transmitted infection at present. Though sexually transmitted (vaginal, anal, or oral sex), penetrative sex is not the only mode of transmission, even skin-to-skin genital contact is enough for transmission. Because this infection has a long lag period from infection to invasive cancer, screening is recommended from the age of 30 years in the general population and 25 years among women living with HIV by WHO. [2] The American Cancer Society, however, recommends screening at age 25 years. Other than the cervix hr-HPV is also responsible for causing vulval, vaginal, anal, penile, and oropharyngeal cancers. Hr-HPV is responsible for approximately 4.5% of all cancers. [5] Among the hr-HPV, genotype16 is responsible for squamous cell carcinoma of the cervix and head and neck, while HPV18 is mostly responsible for cervical adenocarcinomas. The squamous lining of both the cervix and oropharynx, their common locations as an opening in the body that is exposed to the external environment, may be responsible for the persistence of HPV in these regions leading to cancer. WHO recommends a triple intervention strategy (90-70-90) in its venture towards the elimination of cervical cancer and to meet its target by 2030. [6] The triple intervention strategy includes; 90% of girls should be fully vaccinated with the HPV vaccine by 15 years of age; 70% of women should be screened using a high-performance test by the age of 35 and 45; and 90% of women identified with the cervical disease should receive treatment (90% of women with pre-cancer treated and 90% of women with invasive cancer managed). [6] To meet these needs of the hour, especially in the LMICs, many newer tests are being introduced, and newer techniques are being practiced to involve women into the screening.

Techniques

The majority of women in LMICs are socially shy and refuse pelvic examinations required to collect samples for screening, which has led to the evaluation of *urine* as an alternative sample to cervical smear for screening of women for HPV/cervical cancer. HPV can be detected in urine, which may be due to the shedding of virus-infected cells from the cervix or other ano-genital lesions. Many studies have tried to evaluate the similarities of HPV DNA in paired urine and cervical samples from females of all ages, however, these studies show varying concordance levels. [7, 8] There are various ongoing studies to evaluate tests that give the best results and can make urine as an alternative to pelvic examination and increase participation of women in screening. The high population in LMICs makes it difficult for health facilities to cater to the need of women in society. Geographical barriers hinder women from reaching health care facilities and the poor financial status makes women in LMICs prioritize daily wage over visits to health facilities for screening. So if the women cannot come to the health facility for screening, then techniques should be introduced to make the facility reach them. *Self-collection* of vaginal smear for screening has thus been found to be a very efficient way to tackle these problems. In a systematic review done by Braz NS et al., it was noted that in the majority of the studies (17 out of 19), the self-collection method had excellent acceptability. [9] The screening technologies to advance rapid testing—utility and program planning (START-UP) project conducted in India, Nicaragua, and Uganda, demonstrated that 90% of women provided self-collected samples. [10] However, Sowjanya AP et al., in a study showed that the HPV DNA in self-collected samples was 25% to 42% less than physician-collected samples, and the viral load in self-collected samples was 1.4 times lower than the paired clinician collected samples. [11] In a population-based cluster-randomized trial (EMA study), there was a high screening uptake using the self-sampling method, however, the CIN2 + disease detected by the self-collection method was found to be 11% less compared to the clinicians collected samples. [12] Thus, whether self-collection of samples is of benefit to the entire population or should it be limited to the rural areas where screening of women is difficult, is yet to be answered. WHO recommends the use of samples taken by a health care provider or self-collected samples for screening using HPV DNA tests among both the general population and women living with HIV. [2]

Artificial intelligence (AI) is an area where advances in digital imaging and machine learning have given clinicians

the advantage and the hope to improve cervical cancer screening in the present and the future. Deep learning has been applied in slide identification [13], interpretation of colposcopic images [14] and even detection of premalignant and malignant conditions. Cloud-based data storage makes algorithms available from any location and helps users interpret results even without having access to very high-level laboratories. Various devices using AI have been introduced to detect premalignant and malignant conditions of the cervix. FDA has approved MobileODT's Enhanced Visual Assessment system while devices such as "digital cervicography", "TruScreen" which detects the pre-cancerous change by optical and electrical measurements of cervical tissue and "smartoscopy" where smartphones are utilized to evaluate the cervix, are under trial.

Tests

Liquid-based cytology (LBC) is now preferred over Pap for screening due to the benefits of sample collection, transfer, adequacy, clarity, and flexibility. Various large studies have established that the *HPV nucleic acid test* is significantly more sensitive than cytology for screening. WHO recommends using HPV DNA detection as the primary screening test over VIA or cytology in screening and treatment approaches among both the general population of women and women living with HIV. [2] The sensitivity of both tests used together is even better and those screening intervals can be safely extended up to 5 to 10 years after HPV DNA negativity. In a systematic review by Koliopoulos G et al. it was shown that for every 1000 women screened, approximately 20 women will have pre-cancerous changes of which HPV nucleic acid test will be able to identify 18 women while Pap will identify 15 women. [15] The tests are approved by FDA for detection of the nucleic acid are shown in the table (Table1) [16].

These NAATs may be qualitative or quantitative. HPV DNA though very sensitive, the impermanent nature of HPV infection makes its specificity lower than cytology. Hybrid capture used for HPV DNA detection is known to cross-react with the untargeted non-oncogenic types of viruses, thus reducing the test's specificity further. [22] Thus tests have been introduced to detect the viral proteins, E6 and E7, to increase both the sensitivity and specificity of the tests. These two early proteins of HPV (E6 and E7) are directly responsible for the malignant nature of the virus as they target and modulate cellular pathways which are responsible for the regulation of cell cycle, apoptosis and cell polarity, making the virus immortal and innumerable. The viral E6 binds to the p53 tumor suppressor protein and leads to its degradation and also stimulates telomerase activity in keratinocytes. The viral E7 protein binds to the retinoblastoma (Rb) family of tumor suppressor proteins, resulting in the displacement of Rb/E2F complex causing cell cycle progression (Fig. 1). Estimation of E6/E7 oncogene expression using mRNA transcripts has been stated to be more specific in the prediction of risk for cervical cancer than the detection of HPV DNA. Studies have shown that the women with

Table 1 *FDA-approved tests for cervical cancer screening* [16]

Tests (Manufacturer)	Year approved for reflex HPV & co-testing	Year approved for primary screening	Method	Genotypes detected	Sensitivity (%)	Specificity
Digene Hybrid Capture II (Qiagen)	2001	N/A	DNA (non-PCR based): Signal amplification	Qualitative detection of 13 Hr-HPV Types (16,18,31,33, 35,39,45,51, 52, 56, 58,59 and 68)	94.6	94.1% [17]
Cervista HPV HR & HPV 16/18 (Hologic Gen-Probe)	2009	N/A	DNA (non-PCR based): Signal amplification	Qualitative detection of 14 high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) Signal Amplification detects HPV 16 and 18	95.1	90.318 [18]
Cobas (Roche)	2011	2014 (ThinPrep only)	DNA (PCR based); Target amplification	16,18,31,33,35,39,45,51,52, 56,58,59,66, and 68 with genotyping of 16 and 18	100	89.4% [19]
Aptima HPV Gen-Probe)	2011	N/A	mRNA (PCR based); Target amplification	14 high risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68)	94.2	94.5%[20]
Onclarity (Becton Dickinson)	2018	2018 (SurePath only)	DNA (PCR based); Target amplification	16,18,31,33,35,39,45,51,52, 56,58,59,66, 68; simultaneous identification of 16,18 and 45	93.0	87.7%[21]

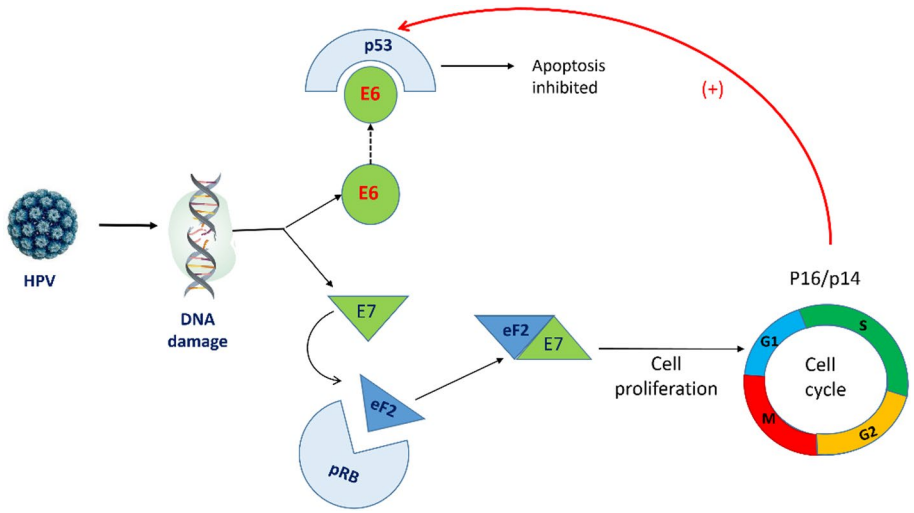

Fig. 1 HPV E6 binds to p53 tumor suppressor protein and inhibits apoptosis. The E7 protein binds to the Rb tumor suppressor proteins, resulting in the displacement of Rb/E2F complex causing cell cycle progression and increased expression of p16

positive HPV E6/E7 mRNA in the cervical samples have a higher risk of progressing to CIN2 in the next 2 years, suggesting that these women with ASCUS or LSIL on cytology should be referred for colposcopy and strict follow-up while women with a negative test can increase the follow-up interval. [23] mRNA-based assays such as NucliSENSEasyQ HPV Test and APTIMA HPV mRNA Assay are being used to quantify the load of viral infections. Rapid diagnostic tests such as the "AV Advantage HPV E6 test" have also been introduced which uses a high affinity monoclonal antibody for the detection of E6 from hr-HPV16, 18 and 45.

Aberrant expression of **miRNA** in the cervical mucus is also being evaluated as a biomarker for cervical cancer and its precursor lesions by utilizing in comprehensive microarray analysis. Integration of the viral genome into the host cell is directly proportional to the viral load and is thus another area being evaluated as a risk factor for the progression of pre-cancer to invasive cancer. HPV E6 and E7 protein interferes with two essential tumor suppressor genes **p53** and **Rb,** and these have been found to be tampered with in poorly differentiated cancers. Detection of **p16** overexpression indicates the presence of the E7 oncoprotein while **Ki-67** expression determines the cell proliferation status. However, **p16/Ki67 dual staining** significantly increases the sensitivity in the detection of CIN2 while maintaining the same specificity when done individually. [24] **C-fos protein** upregulation, **p50 subunit of NF-kB** enhanced expression, **Fra-1** diminished expression, **NOTCH 1** high expression, **Telomerase** activation, **E cadherin** decrease, **Cell Adhesion Matrix proteins,** CD44—involved with tumor growth, spread, and invasion, and **AgNOR** (Silver stained nucleolar organizer regions) where the size and number of the black dots in the nuclei denotes cellular and nuclear activity are all understudy. Squamous cell carcinoma antigen (**SCCAg**), though now used for diagnosis and follow-up of carcinoma of the cervix and lungs, is not useful in screening due to its low sensitivity and specificity. *Next-generation sequencing (NGS)* is an upcoming promising technology and a valuable method for the characterization of HPV genotypes. [25] It is helpful in providing a clearer picture in understanding the mechanisms of carcinogenesis even with poor samples and less sample size. NGS shows higher specificity compared with hybridization methods while higher sensitivity compared to PCR-based assays. It will be a helpful technology in achieving better triage for hr-HPV-positive women.

If women screen positive as per the approved tests by WHO, they can be managed based on resources available (Fig. 2).

Conclusion and Perspective

With the rise of cervical cancer, which is a preventable disease, the global bodies are in search of answers to the reduction in its incidence. There are various trials undertaken to fulfill the unmet needs of the society, especially in the LMICs. It is essential for the clinician to stay in touch with the upcoming tests and techniques and engage in the field of research in order to contribute to the fight against cervical cancer. Clinicians in a low-income setting have a vital role to play in this global fight and having up-to-date knowledge about the recent advances in this field will pave the way for the "cervical cancer-free" tomorrow.

Acknowledgements I would like to thank the TMA Pai endowment fund, ICMR project and INst Biotechnology hub of SMIMS, who are supporting the cervical cancer screening studies being undertaken in Sikkim. Though there was no financial support for this review, but the studies that are being undertaken were an inspiration for writing the review.

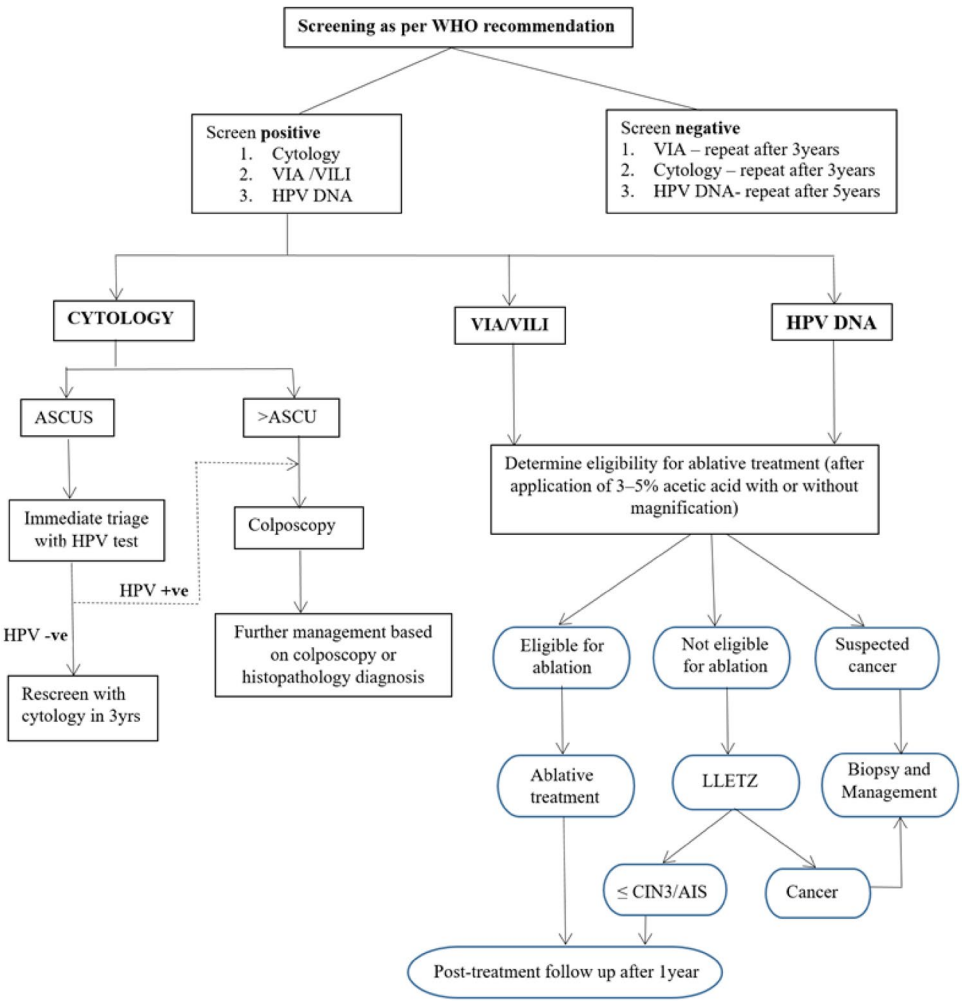

Fig. 2 Management scheme for screen-positive women according to 2021 WHO guideline

Author's Contribution The corresponding author is Dr. Pesona Grace Lucksom, Associate Professor in OBG and a gynae-oncologist at SMIMS, who developed the concept and wrote the paper. The co-authors contributed equally in the collection of articles, writing the paper and reviewing it.

Funding There was no financial support from any specific grant from funding agencies in the public, commercial, or not-for-profit sectors for this review.

Declarations

Conflict of interest The authors declare no conflict of interest.

Human and Animal Rights This review does not involve any research involving Human Participants and/or Animals.

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