

MINI-REVIEW

Multifaceted Usage of HPV Related Tests and Products in the Management of Cervical Cancer - a Review

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Abstract

HPV viruses are integral to the development of cervical cancer. The pathogenesis has been extensively studied. To date, numerous HPV tests and products have been developed and successfully utilized in diagnosis, treatment and prevention of cervical cancer. The HPV DNA test, when combined with other routine cervical cancer screening and diagnostic tests namely exfoliative cytology, visual inspection with acetic acid (VIA) and colposcopy has increased the detection rate of cervical cancer. HPV DNA products could also be measured in other body fluids like urine, lymph node tissue, and serum. HPV association could also be quantified by measuring other parameters like HPV mRNA, viral load, viral integration and methylation status. Vaccination against HPV has been found to decrease the incidence of cervical cancer. Further, therapeutic vaccines for cervical cancer against HPV continue to evolve. All these findings pertaining to HPV could possibly decrease the incidence of cervical cancer in the near future. This review aims to give an overview of the HPV tests and products in use and those under trial currently.

Keywords: HPV - DNA test - triage - cervical intraepithelial lesion - cervical cancer

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Introduction

HPV are a group of heterogeneous DNA viruses which infect the keratinocytes of human skin and mucosa to cause a variety of lesions like skin warts, genital warts, papilloma, cervical intraepithelial lesion and cancer. The virus is transmitted by direct contact, sexual contact and is transmitted to the newborn from the infected mother via vertical transmission. Recently, HPV have been studied extensively though knowledge of HPV associated lesions has been known since ancient times. Hippocrates described HPV in the 4th century as an ulcer transforming into cervical cancer left untreated. Thereafter, in the 19th century, an Italian surgeon Antonio Domenico Rigoni-Stern inferred development of cervical cancer from an infection. George N. Papanicolaou, a Greek scientist, started describing virus related changes in squamous cells. His findings emerged into the Pap smear screening test later. Strauss, in 1949 and Crawford, in 1963 respectively provided electron microscopic structure and physical properties of the virus. Harald Zur Hausen, the Father of Virology, first proposed the relationship between HPV and cervical cancer in 1970. Now HPV is the most elaborately studied virus. Apart from cervical cancer, the virus has been known to be a cause for many other non-genital cancers (Ioannis et al., 2014).

After the establishment of the role of HPV virus in the causation of cervical cancer, the concept has been

extensively used in diagnosis, prevention and treatment of the cancer. The objective of this review is to give a short overview of different HPV related tests and products used in the management of HPV associated cervical cancer.

The HPV virus

The HPV virus is a small, 8-kilo base double stranded DNA virus belonging to the Papillomaviridae family. There are more than 100 different types of HPV virus causing proliferative lesions in human skin and mucosal surfaces. Not all lesions proceed to carcinoma. About 60% of low grade squamous intraepithelial lesions regress spontaneously. In the presence of co-factors like genetic factors, multiparity, oral contraceptive pill use, cigarette smoking, immunodeficiency and other cervical infections like Herpes simplex and Chlamydia; 15% of the low-grade intraepithelial lesions proceed to high-grade lesions. Thirty to seventy per cent of these high-grade intraepithelial lesions were found to evolve into invasive cancers over a duration of ten years. HPV virus is detected in 96.6% of all the cervical cancers (Faruk et al., 2014). The most common types of HPV are HPV 16, 18, 58, 33, 45, 31, 52, 35, 59, 39, 51 and 56 in the order of decreasing frequency. Seventy to seventy six percent of all cervical cancers worldwide are associated with HPV16/18 except Asia where the association is 68-82% (Li et al., 2010). Apart from being implicated in squamous

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cell lesions, HPV viruses are also associated with cervical adenocarcinomas especially HPV18. Eighty nine percent of cervical adenocarcinomas are associated with high risk HPV infection, out of which 54% are of type 18 (Baalbergen et al., 2012).

The HPV DNA test

The presence of HPV DNA in the cervix is detected by two efficient methods i.e. hybrid capture II assay and polymerase chain reaction. These tests are used to detect the presence of high-risk HPV viral infections that are associated with cancer generation. A negative test indicates absence of high risk HPV viral infection. The routine cervical cytology test used for screening cervical cancer has a very low sensitivity. Numerous trials have been conducted to demonstrate sensitivity, specificity and detection rates of HPV DNA. In a comparative study conducted by Shalini et al. (2002) where 4075 women participated, sensitivity and specificity of thin layer Pap smear and HPV DNA test were compared. Both tests were done simultaneously on all participants. Patients with positive test were referred for colposcopic biopsy. Patients diagnosed with Atypical Squamous Cells of Undetermined Significance (ASCUS) and any higher in Pap smear or presence of high risk HPV DNA by hybridization capture method/polymerase chain reaction method were considered positive. The prevalence of Cervical Intraepithelial Neoplasia (CIN)3 or any higher lesion was 3.2%. The sensitivity of HPV PCR (88.2%) and HC assay (90.8%) was higher than thin layer pap smear (61.3%) for identifying CIN3 or any higher lesion. But specificity of thin layer Pap smear (82.4%) was higher than PCR test (78.8%) and HC assay (72.6%) in detecting CIN3 and higher lesions. The study concluded that HPV DNA testing could be used as a screening test for cervical cancer. Ogilvie et al. (2012) did an RCT trial comparing the efficacy of CIN2 detection in ASCUS patients by liquid based cytology and HPV DNA test. Use of HPV DNA test as a potential screening tool was apparent. Othman and Hayati Othman (2014), in their comparative study conducted in Malaysia found increased presence of hrHPV in Pap smears with abnormal cytological findings. They suggested strategizing and implementing HPV DNA test only in cases with ambiguous cytology might improve the cost effectiveness of cervical cancer screening programs in developing nations like Malaysia.

HPV DNA and colposcopy

The HPV DNA test as a screening test could be used to detect more CIN lesions without increasing the colposcopy referral rate. The HART multicentric screening study involved 11083 women older than 30years, with borderline cytological changes in Pap smear. Women with positive high risk HPV test considered positive (825) were randomly divided into two groups. One group was subjected to immediate colposcopy and the other underwent repeat HPV and cytology testing followed by colposcopy after 12 months. The authors concluded that both the methods were found to be equally effective. The

positive women in second group who got negative results during repeat testing at 12 months showed no CIN lesion on colposcopy. Hence, it was inferred that patients with high-risk positive HPV or borderline cytological changes can be followed up for up to 12 months with repeat testing. By doing so, detection rate of CIN lesions could be improved without increasing referral for colposcopy (Cuzick et al., 2003).

HPV DNA test done along with routine Pap smear could reduce colposcopy referral rate in ASCUS patients. Pap smears showing ASCUS may be from CIN2, 3 or invasive cervical cancer. The recommended options for any ASCUS case would be immediate colposcopy, cytology follow up or HPV DNA testing (ASCUS-LSIL Triage Study (ALTS) Group, 2003). A retrospective analysis done on 594 patients with ASCUS who had undergone either immediate colposcopy or colposcopy if HPV positive showed that the detection rate of CIN was higher with colposcopy referred after positive HPV test than with immediate colposcopy (Kececioglu et al., 2013).

HPV DNA and visual inspection with acetic acid

Visual inspection with acetic acid (VIA) is an inexpensive alternative to cytology for regions with low resources. It gives rapid results and needs minimally trained staff. But VIA done alone is not sufficient to diagnose cervical lesions. But HPV DNA testing done along with VIA might provide a good outcome. Jeronimo et al. (2003) conducted a population-based study of cervical neoplasia in Costa Rica involving 5564 women belonging to 25-69 years age group. Subjects were followed up with varying tests like cytology, cervicogram, clinical examination, HPV DNA test and finally referred to colposcopy. The cervicograms of women with positive HPV DNA tests were evaluated to simulate VIA. HPV test and VIA done together gave a sensitivity of 65.5% and specificity of 96.9%. HPV testing done alone gave a higher sensitivity of 81.0% and a lower specificity of 90.8%. Thus, VIA when combined with HPV test could provide better results than cytology especially if a low cost, less time consuming HPV test was provided. Following this method could also be very useful to detect cervical lesions in resource poor areas where cervical cancer screening cannot be done very often. Pimple and Shastri (2014), in a cross sectional cervical cancer study involving 3625 women found that VIA increased the sensitivity and specificity of HPV DNA test in detecting CIN2 lesions. They proved that VIA followed by HPV DNA test could be a cost effective cervical cancer screening strategy in low resource areas.

HPV in the urine

Most women especially in low socio-economic group consider undergoing cervical cancer screening embarrassing and many consider intravaginal examination with a vaginal speculum painful. These factors can contribute to patients' reluctance to subject themselves to screening programs contributing to increasing cancer statistics (Baskaran et al., 2013). So other non-invasive

methods of HPV detection, if available would be appreciable approaches that could increase efficiency of cervical cancer screening. Detecting HPV DNA in urine samples could be a convenient and alternative method for routine invasive method of HPV detection. Majid et al (2014) studied 1000 women; HPV detection was done by PCR method in urine and cervical samples. An 80% concordance was noted. Further in urine samples, the sensitivity for detection of high-grade squamous intraepithelial lesion was 100% with a specificity of 80%. Thus, HPV detection in urine could be a useful alternative screening method. Enerly et al (2013) suggest the use of urine samples in adolescents to study vaccine impact in a large scale. In their review article, where they analyzed about 14 studies that measured HPV in paired urine and cervical samples, they found 75-100% concordance rate between urine and cervical samples in HPV detection.

HPV in lymph nodes

The presence of metastatic deposits in lymph nodes is an important prognostic factor in cervical cancer. The risks of recurrence even in non-metastatic lesions reach up to 15%. A systematic review done on all available data about HPV DNA detection in pelvic lymph nodes in cervical cancer patients found that there was a high correlation between positive HPV DNA and metastasis in pelvic lymph nodes which lead to a hypothesis that HPV DNA detection in pelvic lymph nodes could be a risk factor for recurrence and poor prognosis in cervical cancer (Marco Noventa et al., 2014).

Circulating HPV DNA

HPV DNA integration into the genome of carcinoma cells is a highly specific molecular marker of tumor DNA. Circulating DNA detected using the specific molecular motif is a newly emerging marker, which could be used as prognostic factor for cervical cancers. It could also be used to detect minimal residual disease and recurrence. Circulating DNA might be released more easily from recurrent lesions and metastatic lesions than from primary lesion (Widschwendter et al., 2003). So quantitative estimation of circulating DNA might be used to follow-up the course of the disease. This is supported by a study conducted by Campitelli et al. (2012) on 16 patients with cervical cancer associated with HPV16/18 and for which viral integration motif was already known. Circulating serum DNA for all the patients was analyzed. Out of 13 patients with tumor size more than 20 mm, 11 patients had circulating tumor DNA. Sequential analyses of circulating DNA in two patients showed that concentration of the circulating DNA was related to the tumor dynamics.

HPV DNA and micronuclei

Micronucleus is produced when a chromosome segment fails to incorporate into the nucleus during cell division. It can be readily identified under light microscopy representing genetic damage seen in tissue exposed to carcinogens even before clinical symptoms

arise. Since HPV is known to cause cytological changes in cervical cells, it is hypothesized that micronucleus may be associated with the presence of HPV DNA (Leal-Garza et al., 2002). Cassel et al. (2014) investigated the hypothesis by analyzing 158 cytological smears. Genome amplification of HPV DNA was done for high-risk types 16,18,31,33,39,45 and 59. Micronucleus was detected in 11 out of 20 HPV DNA positive samples and 36 out of 138 HPV DNA negative samples. Presence of micronucleus was higher in HPV positive samples. Also, absence of micronuclei in HPV negative samples may be used to rule out presence of HPV. Thus it was concluded that micronucleus could be used to predict the presence or absence of HPV DNA. Gutierrez et al. (2010) had previously also demonstrated an association between HPV infection and increased frequency of micronuclei in a hospital based case control study.

HPV mRNA

Trials are being done to detect whether HPV mRNA could be used as a better biomarker for cervical cancers. Persson et al. (2014) did a comparative study to compare the efficacy of HPV DNA and HPV mRNA in detecting cervical lesions. Two hundred and nineteen women who were cytologically diagnosed with ASCUS and low grade squamous intraepithelial lesion (LSIL) along with HR HPV positivity involved in a population based cervical cancer-screening program in Stockholm, Sweden were included. HPV mRNA test was done by APTIMA (Gene-Probe Inc., San Diego, CA, USA). The women were followed up for 4 years. During the follow up, 36% of women developed CIN2 or higher lesion. HPV mRNA test was more sensitive (78-100%) in detecting CIN2 or higher lesion but less specific (50%). Thus, they reported that HPV mRNA could be used as a triage test for women with ASCUS but women with LSIL might require more focused tests.

Castro et al. (2013) conducted another trial to test the efficacy of HPV mRNA test and also to determine if it can be used as to triage HPV DNA test. The study included 165 women with HPV DNA positive results. HPV mRNA test (NucliSENS-EasyQ HPV E6/E7-mRNA-assay - Biomerieux) was done on all samples. Women who had cytological abnormalities were referred for colposcopy. 25.8% of the women with ASCUS or LSIL and 89.8% of women with High grade squamous intraepithelial lesion (HSIL) had CIN2 or higher lesion. The sensitivity of mRNA in detecting CIN2 or higher lesion was 81-84% and specificity was 52-80%. Further, mRNA reduced the number of colposcopies done. Women without cytological abnormalities were followed up for 18 months, 34% of which developed CIN2 or higher lesion. mRNA sensitivity and specificity in detecting CIN2 or higher lesion were 83.3% and 89.6% respectively, proving that the mRNA test could be used to triage HPV DNA test. Besides, HPV mRNA test could be used to manage HPV DNA positive women who are colposcopy and histology negative. Rossi et al. (2013) evaluated 673 women with negative colposcopy and positive HPV DNA. They were followed up for 25 months. Seven developed CIN2 lesions.

HPV mRNA test was done earlier or on all the participants. Out of the 7 CIN2 cases diagnosed, 3 were from 82 HPV mRNA positive women, 2 were from 250 HPV mRNA negative women and 2 were from 12 women with invalid mRNA test. This study concluded that HPV mRNA could be a good prognostic marker in the management of HPV DNA positive cases with negative colposcopy and histology. In particular, it could help to reduce the intensity of management in negative patients.

HPV viral load

The HPV DNA test is usually very sensitive but less specific and has a very low positive predictive value (Mayrand et al., 2007). To fill in this gap, quantitative HPV DNA test (viral load) could be used. Studies have been conducted to detect the correlation between viral load and cervical lesions. Schmitt et al (2013) studied viral load of 999 samples with cytological abnormalities i.e. ASCUS, LSIL and HSIL using quantitative real time PCR. After a follow up of 36 months, 79 CIN2 or higher lesions were detected on colposcopic biopsy. Viral load of samples with LSIL and HSIL were higher than other samples with negative cytological results but the difference in mean viral load between the LSIL and HSIL was not significant. The sensitivity for detecting CIN3 was 100% and specificity increased to 95.7%. Thus, viral load testing was found to reduce false positive results and increase specificity and positive predictive value in detecting cervical lesions. Bencomo-Alvarez et al (2012), in their study involving 46 Mexican patients with cervical lesions of various stages also found higher viral load in patients with cervical lesions than in control samples without any cervical lesion. They proposed that viral load could be a potential biomarker for predicting cervical lesions.

HPV viral integration

In cervical cancers associated with HPV, the viral DNA is integrated with the human DNA or it could be seen separately as an episome or both (Woodman et al., 2007). Studies were conducted to detect whether the physical status of the virus could be a prognostic marker for cervical cancer. Das et al (2012) reviewed 270 cervical cancer patients where HPV association was found to be 95%. Amplification of Papillomavirus Oncogene Transcripts (APOT) assay was done to identify viral integration site. Viral integration was noticed in 79% of the cases. Integration preference was frequently found in chromosome loci 1p, 3q, 6q, 11q, 13q and 20q. This study conclusively implies that physical status of HPV viral DNA could be used as a prognostic marker for cervical cancer.

Viral integration occurs at different sites and is randomly distributed all over the host genome. The integration site differs with changing viral structure and the targeted host genome loci (Wentzensen et al., 2004). These integration sites could also be used as biomarker for cancer prognosis. On examination of 57 cervical cancer samples, Xu et al (2013) identified 75 viral integration sites and 39 viral-cellular DNA junctions were identified.

The novel method developed to detect integration sites includes DNA fragmentation and adaptor tagging, PCR enrichment, sequencing, data processing and validation of integration site by junction PCR. The integration sites and junctions were found to have preferential distribution in the host genome contributing to insertional mutation. Thus the developed multiplex strategy method for detection of viral integration site might be very useful and the mapped integration sites might be used as biomarker in the management of cervical cancer.

HPV DNA methylation

Methylated cytosines (meCpG) disrupt transcription factors and also change chromatin configuration. Methylation of CpG sites of HPV DNA plays an important role in carcinogenesis of cervical cancers (Kalantari et al., 2004). Quantifying the amount of methylation might help in the prediction of prognosis of cervical cancer. In a population based case control study that included 273 cervical samples by Mirabello et al (2013), 109 cases were HPV DNA positive with CIN2 lesion. When methylation at 66 CpG sites of HPV genome was quantified in these cases by pyrosequencing assay, 88% of the CpG sites had higher methylation levels. Seventeen per cent of CpG sites with higher methylation levels in non-CIN cases developed CIN2 lesions over a follow up of 3 years. These findings point to the fact that high levels of methylation at CpG sites in HPV genome might be useful in diagnosis as well as to predict future CIN2 cases. Vasiljevic et al (2014) identified 6 genes, methylation of which could be used to detect cervical lesions. Measuring these methylated genes could be a better triage test for HPV DNA test by increasing its specificity.

HPV DNA methylation panel

Many studies have been done to identify methylation frequencies in cervical cancer genes (Wentzensen et al., 2008). If a panel of these methylation targets were identified, they could be used to distinguish lesions that progress to carcinomas and lesions that are destined to regress. Patel et al (2012) used 151 samples and identified a panel of methylation targets. Frequent methylation sites in candidate genes like ESR1, DCC, p16 and LINE1 were chosen to measure methylation levels. Methylation levels were also measured in CpG sites in E6 promotor region and L1 region in HPV genome in 89 HPV positive cases. Lower methylation levels in HPV genome and higher methylation levels in candidate genes were found to be associated with abnormal cytology. Eijsink et al (2011) identified a four gene methylation panel (JAM3, EPB41L3, TERT and C13ORF18) which when combined with HPV DNA test, increased the detection rate of cervical lesions, thereby proving the efficacy of their methylation panel as a triage test for HPV DNA test.

HPV prophylactic vaccine

Two types of HPV prophylactic vaccines are currently available for protection against high-risk HPV infection

Table 1. Summary of HPV Tests, Parameters and their Implications

Tests	Implications	
HPV triage	HPV test before colposcopy HPV test after VIA	Reduces colposcopy referral rate Increases detection of CIN Cost effective
Alternate HPV detection	Urine LN Serum	75-100% concordance rate with cervical samples Prognostic factor for cervical cancer Molecular marker for prognosis of cervical cancer
Other HPV parameters	Micronucleus in cytology mRNA Viral load Viral integration DNA methylation	Predicts HPV infection Triage to HPV DNA test Increases specificity of HPV DNA test Predicts cervical lesion Biomarker for prognosis of cervical cancer Increases specificity of HPV DNA test Predicts cervical lesion

i.e. quadrivalent HPV vaccine against HPV types 6, 11, 16 and 18 and bivalent HPV vaccine against types 18 and 16. Both the vaccines contain L1 protein for antigenicity in the form of virus like particle (VLP) and an adjuvant. The development of VLP in 1991 was a major break through in the development of HPV vaccines. VLP has a virus like structure made of capsomers comprising L1 proteins (Kim et al., 2014). Several studies have been conducted to test the efficacy, safety and immunogenicity of both the vaccines. Harper et al (2004) conducted a trial to test the efficacy of the bivalent vaccine; the efficacy against persistent HPV infection was found to be 95% and efficacy against cytological abnormalities associated with HPV infection was 92% (Harper et al., 2004). Markowitz et al (2013) comprehensively reviewed vaccine efficacy of HPV quadrivalent vaccine in the United States by analyzing the prevalence of HPV infection in the vaccination and pre-vaccination era. The prevalence of HPV infection was found to be low in the vaccination era and vaccine efficacy was found to be 82%.

HPV therapeutic vaccine

It is not only sufficient to prevent HPV infection, owing to the strong association of HPV infection and cervical cancers but is also necessary to discover ways to treat HPV induced cervical lesions. Many immune therapeutic and targeted therapeutic products are under development (Trimble and Frazer, 2009). Live vector vaccines, peptide vaccines, protein vaccines, DNA vaccines and dendritic cell vaccines are some of the therapeutic vaccines currently under clinical trials. Protease inhibitors, histone deacetylase inhibitors, siRNA against E6/E7 proteins are some of the other products under clinical trial targeted against HPV viral products (Knoff et al., 2014).

Conclusions

Current strategies to reduce the global prevalence of carcinoma of the cervix are based on extensive research, which has conclusively linked HPV infection with the aetiopathogenesis of the disease. Social, cultural and resources for both screening and detection have strong implications. Molecular biology has made great inroads in development of predictive markers and prediction of progress of pre-malignant lesions of the cervix. Clearly this link between HPV virus and cervical cancer has paved the way for newer strategies in prevention and

management of pre-invasive and invasive cervical cancers. In this review, various parameters and products related to HPV are analyzed and presented. All these tests should be considered in designing newer strategies in cervical cancer screening programs for detection of early lesions in a cost effective way.

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