Chapter 2

Methodological Algorithms for the Diagnosis of HPV

Maria del Carmen Colin Ferreyra¹,²*, Cristian Fabian Layton Tovar¹, Pedro Perea Garcia¹,²

¹Centro de Investigación en Ciencias Médicas, Universidad Autónoma del Estado de México, México
²Facultad de Medicina, Universidad Autónoma del Estado de México, México

*Corresponding Author: Maria del Carmen Colin Ferreyra, Centro de Investigación en Ciencias Médicas, Universidad Autónoma del Estado de México, Email: mdcolinf@uaemex.mx

First Published March 22, 2016

Copyright: © 2016 Maria del Carmen Colin Ferreyra, et al.

This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source.

Abstract

In this chapter is included method for diagnosing human papilloma based on cell morphology included virus and methods using molecular and immunological techniques. Throughout this review, we compiled information on the main algorithm involved in Human Papilloma Virus (HPV) diagnosis, one of the most diverse and complex diseases that greatly affect the women population in Mexico and worldwide.

Algorithm Based on Cell Morphology Techniques

The cell morphology analysis is one of the most common methodological algorithm to determinate the HPV presence. Nonetheless, HPV infection is high in several countries, being described until now more than 100 genotypes [1].

Pap Smear/Tissues

The diagnosis of HPV can be inferred from morphology, serology, and clinical tools. The Papanicolaou (PAP) is a test designed to differentiate between a variety of cells in vaginal smears for detection of vaginal, cervical and uterine cancer. Furthermore, this procedure is valuable for staining a variety of other bodily secretions and cell smears. The procedure was developed by George Papanicolaou in the early 1940’s. Nevertheless, the sensitivity/specificity test for CIN 2/3 lesions and cervical cancer show a low sensitivity and a high specificity [2]. In women
examined in Durango, Mexico with the Papanicolaou cervical test, a 4.8% prevalence of HPV infection was found, being genotype 16 the most common in that survey [3]. The prevalence of HPV in the men genital tract tends to be similar to that in women with prevalence in external genitalia of 46.4%, 20.8% in the urethra and 12.1% in the meatus [4].

**Colposcopy**

Usually, colposcopy is indicated when the result of a Pap test has changes abnormal. Colposcopy is made to found changes at the vagina and cervix. Most abnormal Pap tests are caused by viral infections, HPV infection, bacteria, fungi (yeast) or *Trichomonas*. However, cellular changes as atrophic vaginitis are natural cervical cell changes related to menopause can also cause an abnormal Pap test. Untreated cervical cell changes that cause abnormal Pap tests may progress the cancerous lesion.

**Visual Inspection**

The sensitivity/specificity test for visual inspection in CIN 2/3 lesions and cervical cancer show a low sensitivity and specificity.

**Algorithm Based on Molecular Techniques**

The diagnosis of HPV infection based on the viral genome detection by molecular biological methods is available from direct samples; the cell culture is difficulty in routine cultivation of these viruses. Molecular techniques are needed to propose new algorithms for diagnostic and therapeutic decisions [5].

**Detection of HPV Proteins**

**Immunohistochemistry**

Immunohistochemistry (IH) is an immunological technique with a spread spectrum of use. Duncan and coworkers have demonstrated that IH can be used to detect HPV in oral cavity squamous cell carcinoma. In this study, the samples of a retrospective cohort of patients with oral squamous cell carcinoma were analyzed by IH to p16 of HPV and compared with results of PCR. These results show that p16 positive by IH (3+) correlates with HPV PCR positivity [6]. This study is an important find to HPV detection in oral carcinoma associated with HPV. Likewise, IH is used to detect HPV in different cell types. The sensitivity in detection of HPV by IH is low and a high specificity; in condyloma and mild dysplasia lesions, HPV capsid antigens were demonstrated by IH in approximately 60% of the lesions, but in several lesions the rate of
Human Papilloma Virus

HPV detection declined markedly with 22%.

**Electron Microscopy**

Electron microscopy is an expensive technique that permits the HPV detection. However, to build and maintain the costs of confocal light microscopy systems is a disadvantage of this method. Broichand coworkers made an analysis performed in 5 patients from large excisional biopsy samples with a fibropapilloma diagnosis. This authors detected spherical viral particles of 40-55 nm in diameter were detected the non-keratinized epithelial cells in 10 specimens examined [7]. Generally, obtained specimens are fixing in a glutaraldehyde solution and processed for routine ultrathin sectioning, the sections of tissue on copper grids are subjecting to amylase digestion of glycogen granules.

**Western Blots**

Western blot (WB) is other several tools for detection of HPV proteins. There are multiple studies that use this method to HPV protein detection. Suchánková and coworkers compare ELISA and western blotting for HPV 16 E7 antibody determination. In this case was found a high degree of concordance between the two tests suggesting that both tests detect the same or similar activity [8].

There are alternative WB test formats that include the analysis of extracts from CaSki cell cervical cancer cell line, because they are expressing endogenous HPV16, using WB16E7 mouse mAb. This is an important method to HPV proteins, considering that E7 has been shown to be the prominent oncoprotein, exhibiting stronger malignant transforming effects than E6 [9].

**Detection of HPV Genomes**

**Direct methods**

**Southern blot**

In the algorithm based on molecular techniques, Southern blot (SB) itself is prone to some errors in interpretation and performance. The precision and accuracy of the assays are several important to establish reference standards useful in this area of molecular diagnostics. SB should give a clinical usefulness of HPV tests and it depends on the different factors mentioned [10].

**In situ hybridization**

The basic principle of the test is based on hybridization nucleic single stranded acids that hybridize to complementary molecules. In this case, the technique employs labeled probe molecules to detect specific complementary target molecules. This technique is a high sensitive method for diagnostic and genotyping of HPV [10].

All cervical carcinomas have at least one high risk HPV [11]. Moreover, the genotypes found in approxi-
approximately 70% of the cases are 16 and 18 [12,13]. From all currently identified genotypes, 40 infect the genital tract approximately, from which 15-20 (genotypes 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) are classified as high risk types because they have been associated with cervical cancer or high-grade of cervical intraepithelial neoplasia (CIN) [11].

There are alternative hybridization test formats that include filters or glass slides as solid supports. In situ hybridization is one of the most sensitive and specific test for HPV DNA. However, it is the most time consuming. Actually, novel methods are promising and some innovative procedures about of routine nucleic acid automatic determination [10].

**Dot blot**

Dot blot hybridization (ViraPap/ViraType), and polymerase chain reaction (PCR) have been compared as the most commonly used HPV hybridization methods with the accepted gold standard–Southern blot hybridization [10]. ViraPap/ViraType agrees with Southern blot, however, this method have a low sensitivity and it only detects seven genital HPV.

**Signal Amplification**

**Hybrid capture**

Hybrid Capture is one of the most widely used tests in the screening of HPV. This technique is based on a RNA of HPV mixture with information about the virus. This technique includes 13 high-risk types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68 giving positive, although not specifically given genotype. An example of this technique is the Hybrid Capture II-test (HC2; Qiagen, Gaithersburg, MD, USA) in this technique is based on the use of the cervical cells, which releases the DNA is denatured and incubated with the RNA probes to allow the RNA-DNA hybrid are detected by adding a substrate which reacts with alkaline phosphatase is formed [14-16].

**Target Amplification**

**PCR**

The polymerase chain reaction (PCR) has been used as a basis for typing techniques and detection of HPV DNA. It is one of the communes and most used methods [17]. The PCR method most widely used is that amplifies the L1 gene encoding structural proteins of the viral capsid between the primers used are MY09 / MY11, GP5 + / GP6 +, because some disadvantages with these primers and the presence of ORF (Open Reading Frame) that can be lost when the virus is integrated into the genome so they could lose amplification and thus give us a false
negative. That is why we have created specific type PCR systems; using oligonucleotide for the E6 and E7 genes, which remain intact during viral integration in the same way, these genes allowed the use of other variations of the PCR as real time PCR, for determining viral load [17-19].

Moreover, to realize PCR techniques, the samples are collected with cytology brush, it must be plastic to have a major performance not degraded DNA amplification by PCR; and this in turn is determined by beta-globin PCR which helps to analyze the quality of DNA for PCR amplification [17]. Regularly, samples processed for DNA extraction are by phenol-chloroform method (now different kit can be used), which detects the presence of HPV DNA by PCR with universal oligonucleotides against the L1 gene by DNA sequencing or the MY09 / MY11 consensus GP5 + / GP6 + that typified by RFLP [20].

Another way to do viral typing is by multiplex PCR with the commercial kit “Kits for Human Papillomaviruses MPCR Maxim Biotech, Inc., following the manufacturer’s instructions, to classify types 6 and 11 low risk, with bands 263 bp and 144 bp, and types 16, 18 and 33 high oncogenic risk, with bands of 601 bp, 360 bp and 413 bp respectively [21].

**Real-Time PCR**

It is a technique in which a sample of cervical secretions in the area of transformation is taken, usually with a paintbrush is disposable, made polyethylene, flexible, which revolves around three to four times to dislodge the cells exfoliated into the vial containing medium for cell collection. Can be used automated systems as Test HPV Cobas 4800 to detect separately genotypes 16 and 18 HPV, where DNA fragments are extended by means of chain reaction (PCR) and hybridization acid nucleic, in order to locate types of high-risk HPV (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 82) in a single analysis to determine the degree of affection cytology causal genotype [22,23].

They have created web-based technology platforms, components and systems in-vitro diagnostics (IVD) and a Lab-on-Point-of-Care Real-time PCR, portable tool for collecting call data from genomic RNA extraction of tumor specimens and / or lymphocytes [24].

Reaction assays TaqMan real time polymerase chain (PCR), using the Sequence Detection System 7900 ABI Prism with 40 reaction cycles (denaturation at 95 °C, annealing and extension at 60 °C) . The quantification has been mainly used to determine HPV 16 and 18 high risk and total human genomic DNA, it is determined in the Alu sequence. Serial dilutions of human genomic DNA, and E7 regions of HPV 16 and 18, of known concentrations were used as standard curves for quantification and acted as positive controls [25].
Algorithm Based Immunological Techniques

Detection of Anti-HPV Antibodies

ELISA Peptides

In the development of anti-HPV, HPV antibodies, pseudovirion (PSV) and encapsulating particles secreted alkaline phosphatase gene (SEAP) are used to measure the infection of cells for 72 - 293TT hours in cell culture supernatant. The SEAP, traditionally measured by Great Escape ™ SEAP Chemiluminescence Kit 2.0 (GE) to potentially reduce costs and increase efficiency. Performance characteristics kit newest chemiluminescence Plus Ultra Test SEAP (Ziva) and GE were compared using the 293TT system. Titration of HPV PSV 16 or 18 have shown noise signals 48 and 72 hours after infection, higher for Ziva in almost all doses beating GE, as it is able to detect SEAP to 48 hours, and when lower numbers of cells were used 293TT [26].

The Elisa DMP2F0 system; R & D systems, Wiesbaden, Germany employs a solid phase monoclonal antibody and an enzyme -linked polyclonal antibody against MMP-2 and MMP-14. The specificity of the antibodies is tested by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) followed by western blot where each test 100ml of supernatant measured, analysis and calibrations are to enhance duplicate. Elisa kit the total protein expression in HNSCC11A, HNSCC14C and CERV196 cells, analyzes the effect of 5-FU and imatinib in HNSCC cell lines and HPV - positive cell lines CERV196 two chemotherapeutic agents are added: imatinib (18μmol / ml) 5-FU ( 5 pmol / ml ) to the cell cultures to determine the expression in the supernatant of cell lines MMP2, like MMP-14; analysis is performed 48, 72, 120, 192 and 240 h after starting the incubation (27). Suppression was observed in Imatinib-associated matrix metalloproteinase in HPV positive patients [28].

The commercial ELISA (Diagnostic DIA.PRO Bioprobes, Milano, Italy) kit was used in the analysis of sera of patients by in vitro tests for IgG antibodies to HPV 6,11,16 and 18; where the optical signals generated in the wells was regularly read at 450 nm with a plate ELISA reader (Optical System Ivymen Model 2100C; Biotech SL, Madrid, Spain). ELISA kit manufacturer provided the formula for calculating the OD450nm cutoff (OD of negative control plus 0.250), used as a threshold for determining serum samples reactive and nonreactive and samples with less than OD450nm cutoff value must be taken as nonreactive and samples with higher OD450nm point, must be taken as positive for IgG specific to HPV antigens in the vaccine [30,31].
VLP

Recombinant virus-like particles (VLPs) are highly immunogenic, may mimic the structure of virions, are better than the attenuated or inactivated virus and could be reversed in infectious form [32]. VLP vaccines have a highly ordered structure that allows repetitive epitope presentations to B cells for activation or potent dendritic cells to stimulate cell-mediated immunity [33]. The VLPs are absorbed by dendritic cells and can present to MHC class 1 [34], which may allow joining the T cells to treat infections or cancer. They can be used both as a preventive (vaccines), as therapeutic [35]. VLPs Human Papillomavirus are able to directly activate dendritic cells may provide the presentation of MHC class I, which can induce immunity cells [36]. VLP chimeras, can act as a transport to carry epitopes of HPV proteins and present them to MHC class I (the merger can be E7 with L1 VLP) [37].

Fused E6/E7

In the diagnosis of cervical cancer, most are based on DNA testing for HPV and Pap smear. The sensitivity of HPV DNA electron microscopic testing is good, but its specificity is relatively low. Currently, European countries such as Norway and Finland, recommend follow-up examinations and tests for HPV E6 / E7 mRNA for the examination of the cervix [38]. Although it has been reported that HPV testing E6 / mRNA E7 has a higher specificity for cancer development, testing DNA-based genes L1 sheds no data on the distribution of infection by specific types of HPV mRNA detected. HPV testing RT-QDX CervicGen is directed against mRNA HPV E6 / E7 oncogenic HPV genotypes 16 HR (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68 and 69) [38-40]).

Figure 1: Methodological algorithms for the diagnosis of HPV.
References


24. Martinelli E, Poli T, Exarchos K, Steger S. Multi- level and multiscale modeling approach for VPH- based prediction of oral cancer reoccurrences. Re-
sults of the FP7 NeoMark project. 2014; 781–784.


