Enferm Infecc Microbiol Clin. 2017;xxx(xx):xxx–xxx

Enfermedades Infecciosas y Microbiología Clínica

www.elsevier.es/eimc

Review article

Microbiological diagnosis of human papilloma virus infection☆

Maria Luisa Mateos-Lindemann[a,*], Sonia Pérez-Castro[b], Manuel Rodríguez-Iglesias[c], Maria Teresa Pérez-Gracia[d]

[a] Servicio de Microbiología, Hospital Universitario Ramón y Cajal, Madrid, Spain
[b] Servicio de Microbiología, Complejo Hospitalario Universitario de Vigo, Vigo, Pontevedra, Spain
[c] Servicio de Microbiología, Hospital Puerta del Mar, Cádiz, Spain
[d] Servicio de Microbiología, Facultad de Ciencias de la Salud, Área de Microbiología, Universidad CEU Cardenal Herrera, Moncada, Valencia, Spain

ARTICLE INFO

Article history:
Received 23 May 2016
Accepted 23 May 2016
Available online xxx

Keywords:
Human papillomavirus screening
Cervical cancer
Human papillomavirus
Human papillomavirus DNA test

ABSTRACT

Infection with human papillomavirus (HPV) is the leading cause of sexually transmitted infection worldwide. This virus generally causes benign lesions, such as genital warts, but persistent infection may lead to cervical cancer, anal cancer, vaginal cancer, and oropharyngeal cancer, although less frequently. Cervical cancer is a severe disease with a high mortality in some countries. Screening with cytology has been very successful in the last few years, but nowadays there are numerous studies that confirm that cytology should be replaced with the detection of HPV as a first line test in population based screening. There are several commercially available FDA approved tests for screening of cervical cancer. A new strategy, based on individual detection of the high risk genotypes HPV16 and HPV18, present in 70% of cervical cancer biopsies, has been proposed by some experts, and is going to be implemented in most countries in the future.

Diagnóstico microbiológico de la infección por virus del papiloma humano

Palabras clave:
Cribado
Cáncer de cuello de útero
Virus del papiloma humano
Prueba ADN

RESUMEN

La infección por el virus del papiloma humano (VPH) es la infección de transmisión sexual más frecuente en el mundo. Este virus ocasiona generalmente lesiones benignas, como verrugas genitales, pero también su persistencia ocasiona procesos malignos, como cáncer de cuello de útero (CCU) y, menos frecuentemente, anal, vaginal y de la cavidad orofaríngea. El CCU es una enfermedad muy severa, con alta mortalidad en muchos países. El cribado de CCU con citología ha tenido mucho éxito en estos últimos años, pero hay innumerable evidencia científica para que sea sustituida por la detección del VPH como prueba inicial. Para esto, hay en el mercado gran cantidad de técnicas, siendo aconsejable utilizar sistemas automáticos y pruebas aprobadas por la FDA. Un nuevo algoritmo basado en la detección individualizada de los genotipos 16 y 18 presentes en el 70% de los CCU ha sido propuesto por expertos y su implantación será inmediata en algunos países.

Introduction

The human papilloma virus (HPV) is a DNA virus belonging to the *Papillomaviridae* family that causes the world's most common sexually transmitted disease. It is generally acquired sexually, but it can also be contracted vertically from mother to child, by contact with the cervical mucosa during delivery, transplacentally and, less often, by horizontal transmission during infancy.[1]

DOI of original article: http://dx.doi.org/10.1016/j.eimc.2016.05.008
☆ Please cite this article as: Mateos-Lindemann ML, Pérez-Castro S, Rodríguez-Iglesias M, Pérez-Gracia MT. Diagnóstico microbiológico de la infección por virus del papiloma humano. Enferm Infecc Microbiol Clin. 2017. https://doi.org/10.1016/j.eimc.2016.05.008
* Corresponding author.
E-mail address: mmateos.hrc@salud.madrid.org (M.L. Mateos-Lindemann).

The prevalence of sexually active women in the Spanish population is 14%, although this can vary according to the age group studied and the associated risk factors. From the age of 40 years the number reduces, to between approximately 5% and 6%.[2]

HPV specifically infects the basal cells of the squamous epithelium of the uterus, and exploits the cellular division of this area to replicate. The typical koilocytes, multinucleated cells and cells with enlarged nucleii form in the upper layer of the epithelium.[1] These cytopathic changes are clearly visible with Giemsa or Pap staining (cytology) in cervical cytology tests. These provide the ideal samples for detecting the virus associated with the cervical disorder it causes.

In most cases, HPV infection is asymptomatic, transitory and can go undetected. In other cases the clinical manifestations are very diverse and range from simple warts and other benign processes to anogenital neoplasias as severe as cervical cancer (CC), anal cancer (AC), penile cancer (PC), cancer of the vagina, and even cancer in other distant anatomical sites such as the oropharynx and the oral cavity (OC). There is now sufficient scientific evidence to show that there is no doubt that the persistence of HPV DNA in the infected cell is an essential condition for the development of cancer.

More than 100 HPV genotypes have been identified, and it is estimated that approximately 40 of these are found in the genital and anal area. Benign manifestations, condylomas and genital warts are caused by non-oncogenic genotypes 6 and 11 (HPV 6, HPV11). These same genotypes also cause recurrent respiratory papillomatosis (RRP), where although rare, a recurrence of papillomas in the respiratory tract can cause death in children and adolescents with the condition.

In 1983, after many doubts and much controversy in the scientific community, zur Hausen et al.[3] (1983) demonstrated the involvement of HPV in the aetiopathogenesis of CC, specifically genotype 16 (HPV16). One year later, genotype 18 (HPV18) was isolated that, along with HPV16, causes 70% of CC worldwide.[4] Precisely because of their level of association with CC, the HPV genotypes are classified into "high oncogenic risk" genotypes (HR-HPV), comprising genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59; "probable/possible high oncogenic risk" (pHR-HPV) (68, 26, 53, 64,65, 66, 67, 69, 70, 73, 82), and "low risk, non-oncogenic" (LR-HPV). VPH6 and VPH11 are the most common of the latter.[5]

There are countless direct tests to detect the virus in cervical samples. Most are based on real-time polymerase chain reaction (PCR), signal amplification and detection of E6/E7 oncogene messenger RNA. However, the Food and Drug Administration (FDA) have only approved 4 tests for use in CC screening.[1] Almost all of them are automated, very reproducible and highly sensitive in detecting the virus in women with premalignant lesions. For all these reasons, it is now agreed that the HPV test should be used as the initial or "first-line" test in CC screening to replace cytology or PAP staining, traditionally used for this purpose but shown in comparative studies not to be very sensitive and to be very subjective. Similarly, considering that HPV16 and HPV18 genotypes cause 70% of CC,[4] all efforts have targeted primary prevention of these specific genotypes (bi- or quadrivalent vaccines adding HPV6 and HPV11) and detection with selective or partial genotyping (individual identification of HPV16 and HPV18, in addition to other HR-HPV), advised in population screening.

CC is the third most common cancer in women worldwide, with some differences in mortality rates according to the country. There is a mortality rate of up to 22.3% in Sub-Saharan Africa. It is estimated that 2511 new cases are diagnosed every year in Spain and there are about 848 deaths, i.e., approximately 2 women every day.[6] These figures are extremely high for a disease that is now completely preventable in any developed country.

Furthermore, HPV not only causes high morbidity and mortality in women, but the malignant processes associated with this virus have increased in recent years as well, principally AC, PC and OCC. Therefore cancers located in the genital areas have increased annually by approximately 3% and OCC by 1%, the latter achieving an incidence of 6.2 and 1.4 per 100,000 in men and women, respectively. These figures might be higher in individuals infected by the human immunodeficiency virus (HIV), men who have sex with men (MSM) and immunosuppressed people, in general. Likewise, HPV16 is the prevalent genotype both in AC and OCC.[7] The screening and vaccination criteria approved for CC also serve for AC, although this terrain is less studied and there are still no validated algorithms to confirm their efficacy.

There are other factors in HPV infection which should be considered, principally the social impact at certain stages of life. CC causes a reduction in a woman's life expectancy estimated at 29 years, considerably greater than breast cancer. Furthermore, adolescents with benign lesions are highly contagious. Therefore, the biological reality is that frequent exposure to HPV infection and reinfection in young people can have serious consequences for their health in the long term.

Virological aspects. Human papilloma virus and oncogenes

HPV belongs to the *Papillomaviridae* family, and is a virus that generally infects the skin and the mucous membranes. It has been classified into HR-HPV and LR-HPV according to its oncogenic capacity.[5] LR-HPV only cause genital warts and condylomas and other benign skin and mucous membrane conditions and association with CC is quite rare. Therefore, from a clinical and screening perspective, only HR-HPV must be detected.

The HR-HPV group comprises genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 (Table 1).[5] Genotype 68 is considered of "probable" oncogenic risk and genotype 66 was previously considered high risk, and therefore they are included in many of the HPV DNA detection tests (HPV test) available on the market.

HPV viruses are small, approximately 50–55 nm in diameter, non-enveloped with an icosahedral capsid formed by 72 HPV capsomeres. Their genome comprises circular, double-stranded, covalently closed DNA, 7500–8,000pb in size. This DNA is divided into: (1) an early E region that codes various structural proteins (E1-E7); (2) a late L region that codes the capsid proteins (L1 and L2), and (3) a regulatory, non-coding region (RNC/LCR), located in direction 5′. The early region (E) represents 50% of the genome; the late region (L), 40%, and the regulatory region (RNC/LCR), 10%.

Relationship between the human papilloma virus and cancer

It has been demonstrated that in the majority of cases HPV infection is a necessary, although not unique, condition for the development of cervical intraepithelial neoplasia (CIN) and cancer. This association is based on various points[8]: (1) the virus is detected in more than 97% of CIN and invasive carcinomas, principally genotypes 16, 18, 31 and 45; (2) HPV infection has a greater relative risk for the development of premalignant lesions, compared with other possible associated risk factors, and the progression of the lesion is related to the type of HPV present in the lesion; (3) a grade 3 intraepithelial neoplasm (CIN3) is highly associated with chronic cervical infection by HPV 16 or HPV18 more than 2 years previously, and (4) there is an association between HPV and carcinomas of the vulva, vagina, PC and AC, and less frequently OCC, and carcinomas of the larynx, oesophagus and respiratory tract.

The integration of HPV DNA in the host cell chromosome is associated with the progression of high grade CIN to cancer.[9] Integration