2nd EDITION

PRACTICAL TRAINING

HUMAN PAPILLOMAVIRUS

PRACTICAL CASES

MODULE 1.

NATURAL HISTORY OF HPV

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INDEX

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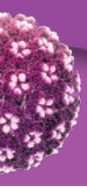
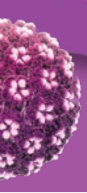


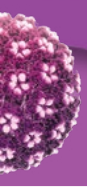
INTRODUCTION

Human papillomavirus (HPV) is an 8,000 base pair double-stranded circular DNA virus encased in an icosahedral protein capsid approximately 50 nm in diameter. The genome can be divided into three different regions. The first encodes proteins expressed early (from E1 to E8, with E6 and E7 most important for inducing cellular transformation). Another region encodes proteins expressed late (L1 and L2, responsible for capsid synthesis). The last region is a non-coding control region that contains regulatory elements.



OBJECTIVES

It is well-established that HPV is the primary cause of cervical cancer. It also has an important etiological role in anal, vulvar, vaginal, scrotal and penile cancer. It is also involved in a growing number of head and neck tumors, and causes genital warts and some other warts. Its disease burden, not only in women but in the general population, is substantial. Therefore, it is important to better understand HPV's epidemiology, routes of transmission, and interactions with epithelial cells.



1. EPIDEMIOLOGY

HPV infection is the most prevalent known sexually transmitted infection in humans. In the US, a prevalence study from 2014 revealed the presence of high-risk HPV in 20% of women between 18 and 69 years of age. Practically the entire sexually active population would have come into contact with the virus at some point in their lives[1].

In Spain, it is estimated that approximately 2 million women are HPV-positive, of which 300,000 may present mild or more severe cytological abnormalities, with a diagnosis of approximately 2,500 new cases of invasive cancer each year.

There are 35-40 distinct genotypes of HPV capable of infecting the genital tract. Types are classified as high-risk (HR), low-risk (LR), and probable high-risk (PHR). Here, we show Bouvard's classification[2]:

Risk	HPV Serotypes
HR	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68
PHR	26, 53, 73, 82
LR	6, 11, 40, 42, 43, 44, 54, 61, 62, 70, 71, 72, 81, 83, 84, 85, 89

Notwithstanding, it is important to mention the recently proposed classification by Cuzick[3], according to which types 16, 31 and 33 should be considered very high risk, while types 39, 56, 59, 66 and 68 should be considered of intermediate risk.

According to Cuzick, types 18 and 45 do not have a very high predictive value for developing CIN2 and are more closely related to invasive cancer and lesions of the endocervical canal.

The high-risk serotypes are those normally more closely related to invasive pathology. These are responsible for practically all cervical cancers, between 70-90% of vaginal and anal cancers, 40% of vulvar cancers and 20% of oropharyngeal cancers[4]. Only types 16 and 18, the most important, are related to 70% of cervical cancers.

The low-risk serotypes are rarely involved in malignant pathology, although they are commonly seen associated with genital warts and recurrent respiratory papillomatosis (RRP) in children and adolescents.

The risk of infection by HPV:

» Increases with the number of partners, for both the woman and her respective partner(s). This is likely the primary risk factor for higher viral prevalence.

» Is inversely proportional to age, with greater prevalence in young women, at around 30% near 20 years of age. The incidence decreases and plateaus around 12% at 30 years of age, after which point the drop is much more gradual. Approaching 65 years of age, there is a slight upturn in incidence, probably related to acquiring new partners. However, some authors speak of the reactivation of latent infections.

» Increases with tobacco use.

» Is reduced by using a condom, though not eliminated.

» Is reduced in the case of circumcised male partners, given their lower viral prevalence.

» Increases with the practice of anal sex, both for homosexual males as well as heterosexual partners.

» Increases in the presence of other sexually transmitted infections (STIs), especially *Chlamydia trachomatis*.

2. TRANSMISSION

HPV is acquired through direct contact between the skin and/or mucosa. It is a cellular virus and its ability to survive outside the body is very limited. Acquisition through contact with inert surfaces such as public bathrooms, is not documented. However, sex toys used directly during intimate relations can become contaminated and act as vehicles of transmission.

Thus, sexual encounters are the primary cause of HPV infection. Transmission has been documented via oral sex, though less frequently than vaginal or anal intercourse.

2.1. Aspects of transmission through intimate contact

According to a study by Hernández *et al.*, spread is more common from female-to-male than from male-to-female. Thus, an overall rate of transmission from penis to cervix was calculated at just 5% per person-month, while the overall rate of transmission from cervix to penis reached 17.5% per person-month. Female-to-male transmission is highest, however, in anal intercourse (47% per person-month). This is even higher than the incidence of spread from the cervix through vaginal intercourse (28% per person-month) to the entire male genitalia (penis and scrotum). Likewise, a greater incidence of male-to-female spread was documented in anal sex than in vaginal intercourse. There was also bidirectional viral transmission through hand/penis contact, more frequently in the female-to-male direction. This work found no spread through semen. Interestingly, an episode of viral clearance in the female anus was observed, with subsequent reinfection after another anal copulation, which demonstrates the virus' capacity to reinfect the same epithelium after its clearance. Hernández, on the other hand, found no evidence of spread through oral sex, though the study did find self-infection between areas within the same individual[5].

As mentioned previously, the use of a condom offers very limited protection, simply because it does not fully cover the genital area of either sex. It always leaves the anus, vulva, base of the penis and scrotum exposed. When these epithelia make contact, viral transmission can occur. According to Hernández's findings, the incidence of spread exclusively from the cervix to the scrotum is only 50% lower than that quantified in the penis.

According to Gayón, the use of a condom decreases the risk of HPV infection by 70% in women. This risk drops to 50% if condoms are only used in half of sexual encounters[6].

A recent systematic review of other longitudinal studies found that in at least half of these, the use of a condom provided statistically significant protection against HPV transmission[7].

Thus, the use of a condom continues to be useful in reducing the risk of transmission, as well as the risk of progression of HPV-induced lesions. Condoms reduce that risk, prevent transmission of other STIs, and serve as contraceptives. Condoms continue having a fundamental role in any casual sexual relationship, or among couples where either partner has other sexual partners.

2.2. Vertical transmission

Maternal-fetal vertical transmission is very controversial, both in terms of the level of actual risk for the fetus, and in discerning the primary route of transmission.

Traditionally, it has been considered that passage through the birth canal is the route of maternal-fetal HPV transmission. However, several studies call this view into question. Aside from neonatal oropharyngeal secretions, the virus has also been found in cord blood and even the placenta. In addition, some works indicate that elective cesarean procedures do not protect against such transmission, since there are no significant differences in detecting HPV in newborns of patients who gave birth vaginally or via cesarean.

A 2013 study by Lee *et al.* noted that 75% of the viruses detected in newborns coincided with strains found in the mother during the first trimester. However, the mothers had cleared the viruses later in gestation, which indicates that transmission probably occurred early[8]. Other work showed the presence of HPV in amniotic fluid of pregnant women with altered cytologies[9].

Furthermore, it is not clear whether the detection of viral presence in newborns has long-term consequences. This could overestimate the possibility of pathology. As we well know, simple viral presence does not imply active infection.

Though there are works that show that this presence is cleared between 6 and 12 months after birth[10], other authors detected HPV in those newborns months and years afterwards[11]. Some oropharyngeal malignancies are due to HPV. If viral presence persists and the infection becomes latent, vertical transmission cannot be ruled out of having a role in tumor pathogenesis. New studies are needed in this area as a result.

While discussing vertical transmission, RRP deserves a special mention, as it presents in infancy or, at the latest, in adolescence, with a benign picture that consists in the appearance of papillomas, particularly in the larynx, that have a tremendously high tendency to recur, despite destructive treatments. RRP behaves like a chronic condition with frustrating management. It almost always tends to appear before 5 years of age, though its incidence, luckily, is fairly low, with just 4 new cases per year per 100,000 inhabitants.

In about 90% of cases, it is due to low-risk genotypes 6 and 11, though in 5% of these, serotype 16 is detected.

In the case of vulvovaginal papillomatosis during pregnancy, elective cesarean section seems to improve the likelihood of avoiding RRP, but does not fully prevent it. RRP is 4.6 times more likely in children born vaginally. The incidence is also 2.5 times greater in children of mothers under 20 years of age[12].

The general consensus is not to electively use the cesarean for delivery in the case of vulvovaginal condylomatosis. First, because the incidence of RRP is very low and the complications of the cesarean are higher in frequency. Second, because as we have seen, the protection offered is very limited.

2.3. Iatrogenic spread

Some work has revealed potential contamination with HPV of transvaginal probes in up to 25% of cases in healthcare settings. This includes up to 3% after employing quaternary ammonium-based disinfectants. However, there is no evidence of nosocomial spread by this or other means of gynecological examination[13].

2.4. Spread through inhalation

The surgical profession has been concerned for some time about staff exposure to HPV from fume inhalation during laser or conical cauterization and vaporization. Though there are no randomized studies for this, a systematic review of the literature by the US Centers for Disease Control and Prevention (CDC) was performed, reviewing 25 studies related to this exposure. 7 of these studies revealed the presence of HPV-DNA in the laser fumes and one in the cautery, though it was impossible to determine if this material was viable and capable of causing infection. Three cases were reported, two in surgeons and one in support staff, involved in laser vaporization of anogenital condylomas and rectal lesions, who developed laryngeal papillomatosis. There is no evidence of an increase in these pathologies among these personnel relative to the general population. In any case, to reduce possible exposure, it is recommended to always have good operating room ventilation, use surgical respirators and fume extraction and aspiration equipment[14].

3. INFECTION

HPV infection is a prerequisite for the appearance of cervical cancer. Alterations, cellular atypias, and invasive pathology frequently occur in the cervix. Indeed, something must occur here to make it especially sensitive to HPV-induced changes. That peculiarity is the existence of the so-called "transformation zone." This zone is the frontier between two epithelia: (1) the stratified squamous epithelium of the vagina, which is particularly resistant, and (2) the more delicate, simple columnar glandular epithelium of the endocervix. It is a very unstable zone with high mitotic activity related to the mechanisms of metaplasia and re-epithelialization. This is why HPV has greater ease of integrating itself into these cells' genomes. Additionally, this area can have more small, raw areas (erosions) that make it much more likely that the virus can come in contact with basal cells.

Anal intercourse can cause epithelial damage and erosions, through which the virus can enter and provoke cellular alterations.

In any case, HPV penetrates deeply into the basal layer, where it provokes infection. There, it has a very high likelihood of regression and a limited capacity for progression. It is estimated that around 70% of HPV infections are cleared during the first twelve months and around 90% during the first two years.

When the virus persists for 18-24 months or more, the possibilities of regression drop drastically, especially in older (perimenopausal) patients, from 20 to 35%.

At the clinical and epidemiological level, we can divide HPV-induced cellular alteration into 4 stages:

1. **Normal cell.** Before infection or having cleared infection.

2. **Low grade change.** This stage has a high capacity for regression to normalcy, and should not be considered premalignant. In young people, we can expect a regression rate of 90% and of 55-60% over age 30.

3. **High grade change.** This stage has a considerably lower capacity for regression, from 30-50% depending on age. This stage should already be considered a premalignant process.

4. **Invasive carcinoma.**

Virus clearance in cell changes is related to the regression of lesions and vice-versa. Viral persistence is required for progression toward high grade lesions and invasive cancer.

HPV and its impact on the host

- Induction of low antibody titers
- Low macrophage activation by the viral capsid
- Induction of limited immune memory
- Limited induction of inflammation
- Weak stimulation of the adaptive immune system

There is new evidence that increased vaginal microbiota (VM) diversity, combined with a lower relative abundance of *Lactobacillus*, fosters the persistence of HPV, as well as the development of precancerous cervical lesions.

The VM is classified into five types: Types I, II, III and V are characterized by the dominance of *Lactobacillus crispatus, L. gasseri, L. iners* and *L. jensenii,* respectively. Type IV does not normally include *Lactobacillus* and is rich in strict anaerobes, among them *Gardnerella, Megasphera, Sneathia* and *Prevotella*. This is the microbial basis of bacterial vaginosis, which has been linked to a higher incidence, prevalence, and persistence of HPV infection, as well as the development of intraepithelial cervical lesions[15].

Recently, a study has evaluated the impacts of VM changes on the genesis of intraepithelial lesions and has proposed that these alterations could be used to monitor progression of intraepithelial lesions toward cervical cancer[16].

It is interesting to note that though host immunity almost always ends up clearing the virus, HPV is quasi-“silent” to the immune system. Because it is exclusively intraepithelial and does not destroy host cells, it better evades the innate immune response.

It is noteworthy that when HPV infects the genital tract, it is possible to find different lesions in different stages of progression. Each lesion is independent and has its own development. Even when a person is infected with multiple strains, we might find a unique viral type in each lesion. A single strain may even be responsible for all the lesions at different stages. Other strains could be present without causing active infection.

3.1. Molecular basics

Epithelial cells closer to the surface tend to do most of the amplification of viral DNA. These cells transcribe the early oncoproteins E6 and E7 at very high rates. However, being highly differentiated cells, they have lost the capacity to divide, so oncogenic transformation does not occur. Right away, the transcription of the late proteins L1 and L2 of the capsid occurs and the viruses are released rapidly. This is mainly the infectious model of low-risk viruses that provoke the appearance of genital warts and condylomas.

In basal cells, viral DNA amplification is lower, reducing mutation appearance. However, the expression of oncoproteins E6 and E7 significantly increases genomic instability, albeit slowly.

There is therefore a hypothesis that high-risk HPV is capable of keeping its cell infected in a “stem cell”-like state. In that way, the virus integrates itself into the cell genome in order to establish that dangerous persistence in cells with high mitotic capacity. Meanwhile, low-risk viruses rapidly produce viral progeny to maximize their transmission in large productive lesions formed by mature cells[17].

The integration of the high-risk HPV genome into the host DNA is thought to be due to the instability in the host cell facilitated by E6 and E7. These two genes are integrated into said DNA. The rest of the viral genes are discarded or simply not transcribed.

In the case of the E6 and E7 proteins, the primary inducers of oncogenesis, their action is complementary. They need each other mutually to induce the appearance of changes that eventually lead to cancer.

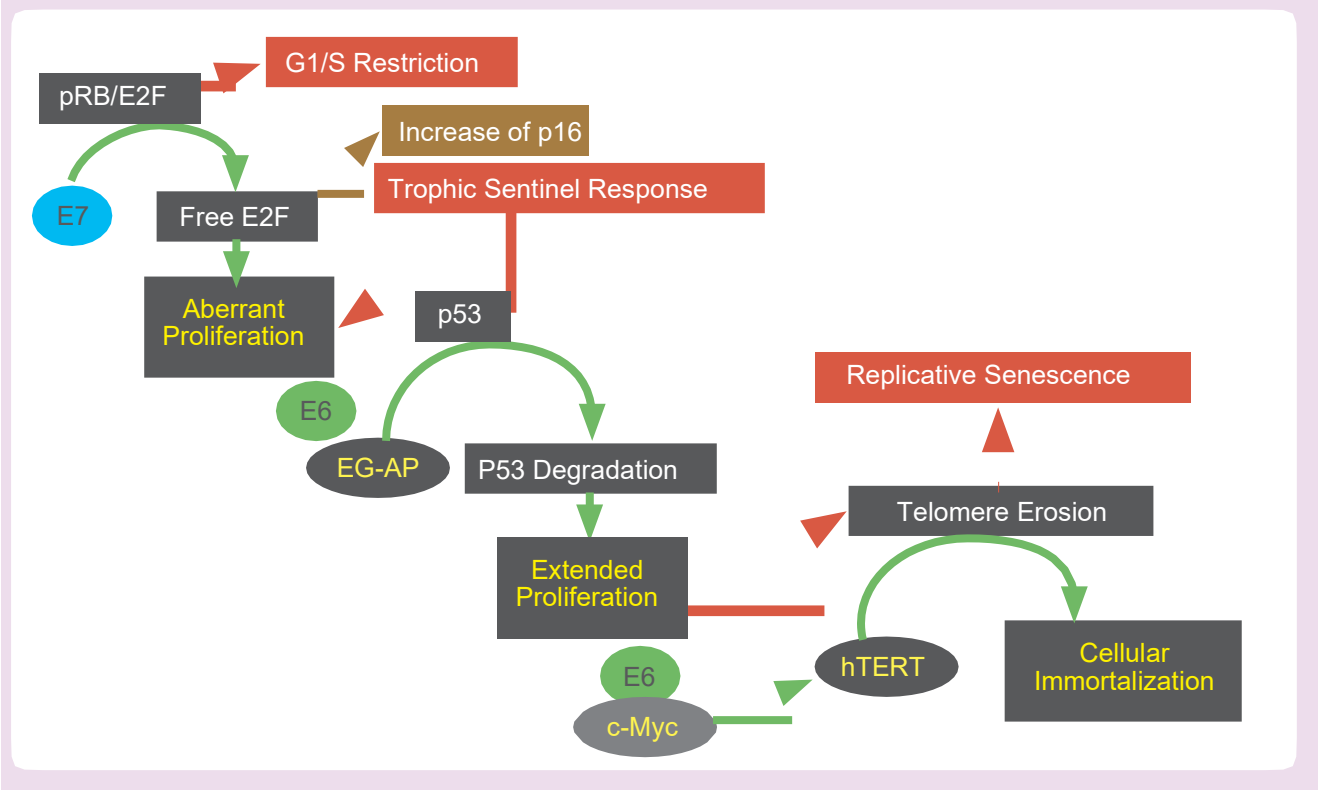
In the uninfected cell, the retinoblastoma protein (pRB) is bound to the E2F family of transcription factors, thus controlling cell replication. But the E7 oncoprotein is capable of binding to pRB, which leaves E2F free and the cell enters the S phase of replication. This unleashes the production of p16 in a futile attempt to inhibit the process again, so immunohistochemical stains for p16 are a good indicator of proliferation in HPV-induced lesions.

But this is not the sole action of E7, which also alters cell metabolism. It delays basal cell differentiation, converting it into a “stem cell” reservoir, which maintains a stable pool of infected cells. E7 is a potent mutagen that induces abnormalities in the centrosome of chromosomes during mitosis, provoking aberrations in these, deletions, and losses of genetic material.

The induction of replication and appearance of genetic aberrations kick-start a trophic sentinel response, which can provoke cell differentiation, senescence and apoptosis. This response is mediated by p53[18]. But E6 is capable of binding to E6-AP, a cellular protein, forming a complex that stimulates the rapid degradation of p53. It can also interfere in the transcription of p53, binding to other proteins.

Each time a cell divides, erosion of the terminal chromosomal telomeres can occur. To avoid this, telomerase proteins act upon them and are increased in tumors. The over-expression of the hTERT subunit of telomerase indicates an increase in the cell's life expectancy and brings the cell closer to immortality. E6 is capable of interacting with the c-Myc terminals, capable of stimulating these hTERT subunits[19] (Fig. 1).

Figure 1. Schematic of critical steps in HPV-induced carcinogenesis. Adapted from *Münger* et al.



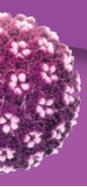
3.2. Cofactors

HPV is the factor required for cervical cancer, but there are other cofactors capable of increasing the risk of progression:

» **Tobacco.** The mutagenic role of tobacco and its presence in the cervical mucus are well-known. There is a well-founded association between tobacco use and the risk of progression[20].

» **Condom use.** The use of a condom reduces the duration of HPV infection and its likelihood of progression.

» **Use of hormonal contraceptives.** It is recommended to discontinue their use, unless the risk/benefit justifies it. It is probably the cofactor that is most debated over the years and, of the three listed, the least influential.

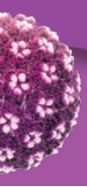


4. CONCLUSIONS

HPV is a highly prevalent infection and affects practically the entire sexually active population at some point. Its disease burden is high, even in males, with serotypes 16/18 most commonly involved in severe pathology.

The primary route of transmission is sexual. This does not mean exclusively through intercourse, as contact between contaminated epithelia is sufficient. Therefore, condom use does not provide complete protection. Vertical transmission is well-documented, though its long-term significance is disputed, and its manifestations are rare. Other types of transmission such as through gas inhalation or iatrogenic contamination require further research.

The majority of minor infections and manifestations are cleared spontaneously. Viral type and changes to the vaginal microbiota may influence the clearance process. The virus has a high capacity for avoiding the innate immune response. Oncoproteins E6 and E7 are responsible for cellular persistence and immortalization processes. Tobacco use is the most significant cofactor contributing to the appearance of severe lesions.



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