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REVIEW ARTICLE

Human Papilloma Virus Infection, Epidemiology and Vaccine Status

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Abstract

Cervical cancer caused by Human Papilloma Virus (HPV) is being the second most common cancer among women and majority of cases occurs in developing countries and it is the leading cause of cancer mortality in women. Being so, it is very important to understand about the virus types and its association in cancer causing. The knowledge on the infection of the high-risk human papilloma virus prompted the exploration of its utility in cervical screening. It is also equally important to understand the disease diagnosis and its epidemiology pattern worldwide. Currently there is Virus Like Particle (VLP) based prophylactic vaccines available against HPV types 16 and 18 which has shown high titers of neutralizing antibodies. There are also emerging second generation recombinant HPV prophylactic vaccines based on both HPV core proteins (L1 and L2) are in the clinical study focusing on low cost manufacturing and broad protection concepts. Here, in this review we have discussed about the prevalence of the disease, infection, disease epidemiology and current vaccines.

Keywords: Cervical cancer, human papilloma virus, virus like particle, prophylactic vaccines.

Introduction

Cervical cancer is the second leading cause of cancer deaths in women worldwide. Cervical cancer occurs when abnormal cells on the cervix grow out of control. The cervix is the lower part of the uterus that opens into the vagina. Cervical cancer can often be successfully treated when it is found early (Eifel *et al.,* 2011). The primary factor in the development of cervical cancer is infection by the "Human papilloma virus (HPV)". HPV is one of the most common sexually transmitted diseases in the world. It is instantly known that cervical cancer is a result of persistent infection with high risk type HPV (Kim *et al.,* 2007). Persistent high risk human papilloma virus (hrHPV) infection is necessary, but alone is not sufficient, for the growth of cervical cancer (Van *et al.,* 2011). Fifteen of the >120 known HPV genotypes are considered high risk (Clifford *et al.,* 2003; Munoz *et al.,* 2004; Joura *et al.,* 2014), but HPV16 alone causes half of all cases of cervical cancer and predominates in other HPV associated anogenital cancers and those of the oropharynx (Bosch *et al.,* 2013; Alemany *et al.,* 2014; Alemany *et al.,* 2015). The hrHPV cause ~5% of all cancer deaths globally, simply the greatest burden is among women who presently are not achieved by effective cervical cancer screening programs, such that >85% of cervical cancers occur in low resource settings in the evolving world (Van *et al.,* 2012; Wang *et al.,* 2013).

Human Papilloma Virus (HPV)

Human Papilloma Virus (HPV) is a DNA virus from the papilloma virus family that is capable of infecting humans. Most HPV infections are subclinical and will cause no physical symptoms; however, in some people subclinical infections will become clinical and may cause benign papillomas such as warts (verrucae) or squamous cell papilloma, premalignant lesions that will drive to cancers of cervix, vulva, vagina, penis oropharynx and anus (Stanley *et al.,* 2012). Totally >120 HPV genotypes have been identified so far that mainly cause benign epithelial papillomas on skin or mucosa (DeVilliers *et al.,* 2004). So-called "low-risk" (LR) types, mostly HPV6 and 11, induce benign mucosal warts (condylomataacuminata). Persistent infection with "high-risk" (HR) types, mainly HPV16 and 18, are associated with almost all cervical cancers (Walboomers *et al.,* 1999). A significant number of HPV types show a tropism for the skin or cutaneous epithelium, at body sites other than the anogenital region (Table 1).

HPV Genome Organization

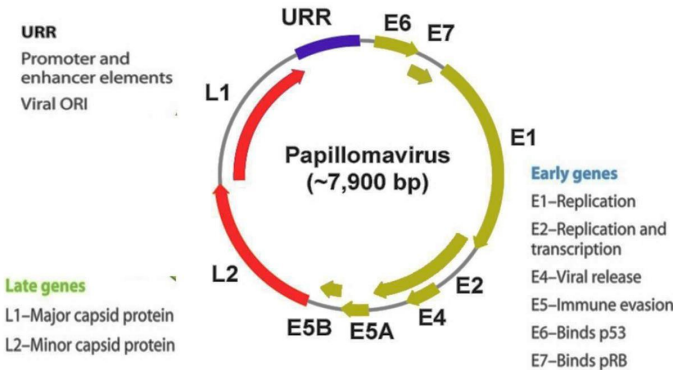
Virus particles consist of about 7900 base-pairs (7.9 kbp) long circular DNA molecules wrapped into a protein shell. The HPV genome can be functionally divided into two regions: Upstream Regulatory Region (URR) and Open Reading Frames (ORFs). URR does not code for proteins, but contains cis-elements required for the regularization of the gene expression, replication of the genome, and its packaging into virus particles. ORFs can be divided into the Early Region (E), necessary for the replication, cellular transformation and the control of viral transcription, and Late Region (L) that codes for the capsid proteins that comprises the outer coat of the virus (Munoz *et al.,* 2006) (Fig. 1).

Table 1. Human papillomavirus types and clinical manifestations (Bonnez *et al.,* 2000).

Clinical manifestations	**HPV type***
Plantar warts	1, 2, 4, 63.
Common warts	2, 1, 7, 4, 26, 27, 29, 41, 57, 65, 77, 3, 10, 28.
Flat warts	3, 10, 26, 27, 28, 38, 41, 49, 75, 76.
Other cutaneous lesions (e.g., epidermoid cysts, laryngeal carcinoma)	6, 11, 16, 30, 33, 36, 37, 38, 41, 48, 60, 72, 73.
Epidermodysplasia verruciformis	2, 3, 10, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23,24, 25, 36, 37, 38, 47, 50.
Recurrent respiratory papillomatosis	6, 11.
Focal epithelial hyperplasia de Heck	13, 22.
Conjunctival papillomas/carcinomas	6, 11, 16.
Genital warts (condyloma acuminatum)	6, 11, 30, 42, 43, 45, 51, 54, 55, 70.
Low-risk cervical intraepithelial neoplasia	6, 11, 16, 18, 31, 33, 42, 43, 44, 45, 51, 52, 74.
High-risk cervical intraepithelial neoplasia	16, 18, 6, 11, 31, 34, 33, 35, 39, 42, 44, 45, 51,52, 56, 58, 66.
Cervical carcinoma	16, 18, 31, 45, 33, 35, 39. 51, 52, 56, 58, 66, 68,70
Other genital carcinomas (vagina, vulva, penis and anus)	16, 18, 31, 45, 33, 35, 39. 51, 52, 56, 58, 66, 68,70

*Order indicates the relative frequency; Bold type indicates most frequent association.

Fig. 1. Schematic presentation of the HPV genome. The non-coding region is the upstream regulatory region (URR). The open reading frames (ORFs) encode the early (E), and late (L) viral proteins.



Source: Munoz *et al.* (2006).

Within the Early Regions (E) it is possible to distinguish different genes with specific functions. E1 and E2 genes have an important role in basal DNA replication. During viral persistence, the immune system keeps the infection in this state. E2 participates in the regulation of LCR (low-copy repeats) transcriptions and decreases the expression of E6 and E7. The E4 gene codes for one family of small proteins involved in the transformation of the host cell by producing alterations of the mitotic signals and interacting with keratin. E5 decreases intercellular communication and isolates the transformed cells and interacts with the growth factor receptors and encourages cellular proliferation. It also stimulates the expression of E6 and E7. E6 is oncogenic, stimulating the growth and transformation of the host cell by the inhibition of protein p53's normal tumor-suppressor function. E7 also acts as an oncogene, inducing cellular proliferation by inhibition of protein pRb.

Within the Late Region (L), it is possible to distinguish the L1 gene, which codes for the major capsid protein and can form virus-like particles and L2, which codes for the minor capsid protein (Jones and Wells, 2006).

HPV Life Cycle

HPVs are perfectly adapted to their natural host tissue, the differentiating epithelial cells of the skin or mucosa and exploit the cellular machinery for their own purposes. HPVs are undergoing a complete life cycle only in the fully differentiated squamous epithelium. These viruses infect the basal cell layer where they establish their small double-stranded DNA genome, as a circular extra-chromosomal element or episome in the nucleus of infected cells (Fig. 2). Following the entry in the suprabasal layer, the viral genome replicates and in the upper layers of epidermis complete viral particles are released (Doorbar, 2005). The existence of the viral genome in the infected cell is central to the life cycle of papilloma viruses and their associated pathologies. Maintenance of the viral genome requires the activity of E1 (the replicative helicase of papilloma virus) and E2, the two viral proteins necessary for replication of the HPV genome in conjunction with the host cell DNA replication machinery. As an initiator protein, E1 acts both as a DNA binding protein to recognize the viral origin of DNA replication and subsequently as a helicase to unwind the origin and the DNA ahead of the replication fork. In lesions containing HPV episomes, the viral E2 protein directly represses early gene expression as part of a mechanism to regulate copy number. Integration of viral DNA usually disrupts E2 expression, leading to the deregulated expression of early viral genes, including E6 and E7. The expression of viral gene products is closely regulated as the infected basal cell migrates towards the epithelial surface. Genome amplification, which is necessary for the production of infectious virions, is prevented until the levels of viral replication proteins rise, and depends on the co-expression of several viral proteins. Viral persistence leads to clonal progression of the persistently infected epithelium.

Fig. 2. HPV Life Cycle (Adapted from Doorbar, 2006).

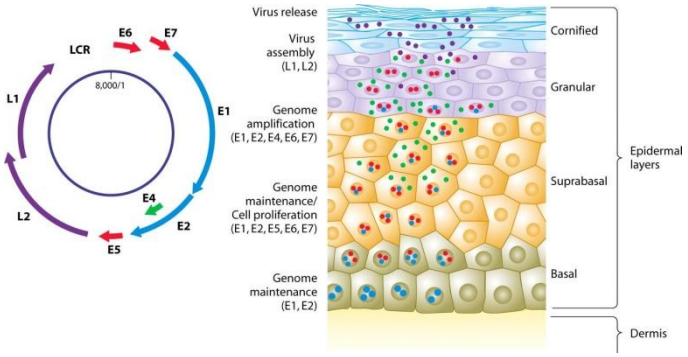
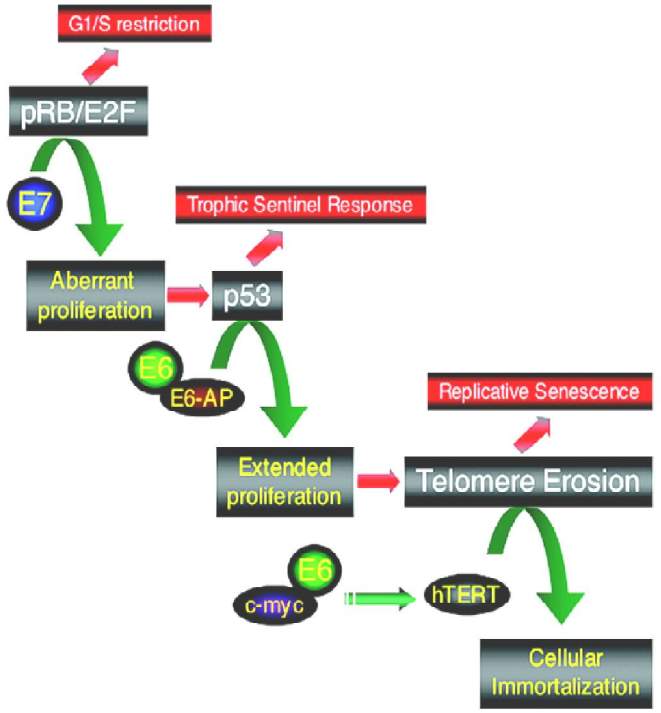


Fig. 3. Schematic outline of the critical steps of high-risk HPV induced carcinogenesis. Inactivation of the catalytic telomerase subunit hTERTconstitutes a subset of the steps that have been shown to be necessary for the generation of fully transformed human epithelial cells *in vitro* (Karl *et al.,* 2004).



Events which are still not completely understood lead infected cells to malignant transformation. Tumor formation is not an inevitable consequence of viral infection; it rather reflects the multi-step nature of oncogenesis where each step constitutes an independent (reversible or irreversible) genetic change that cumulatively contributes to deregulation of cell cycle, cell growth and survival (Bosch *et al.,* 2008).

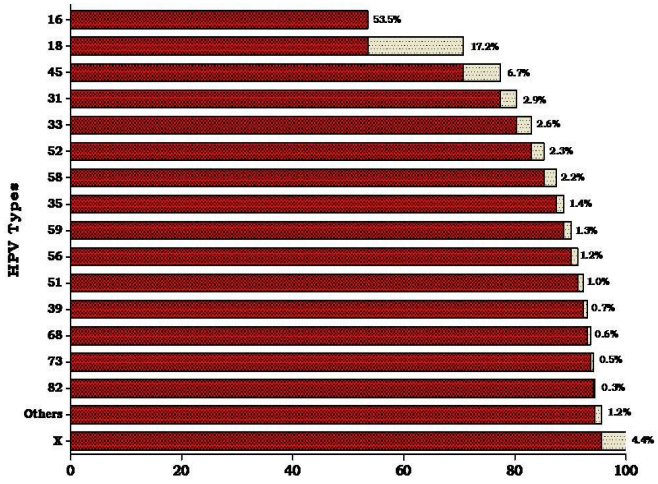
Mechanisms of HPV-Induced Oncogenesis

One of the key events of HPV- induced carcinogenesis is the integration of the HPV genome into the host chromosome. HPV genome integration often occurs near common fragile sites of the human genome (Thorland *et al.,* 2003). But there are no apparent hot spots for integration and no evidence for insertional mutagenesis (Ziegert *et al.,* 2003). The continued combined expression of high-risk HPV E6 and E7 proteins in cervical cancers causes inactivation of the pRB and p53 tumor suppressor pathways and induces telomerase activity.

HPV and Genital Cancer Epidemiology

In spite of screening efforts, cervical cancer remains the second most common cancer in worldwide. In developed countries, it is the most common cancer in young women. HPV DNA is detected on average in 10% of normal cytology and almost at 100% of cervical cancer specimens (Fig. 4). HPV 16 and 18 account for at least 70% of cancers worldwide. They both induce persistency and progression at a higher rate than other high-risk oncogenic types (Fig. 5).

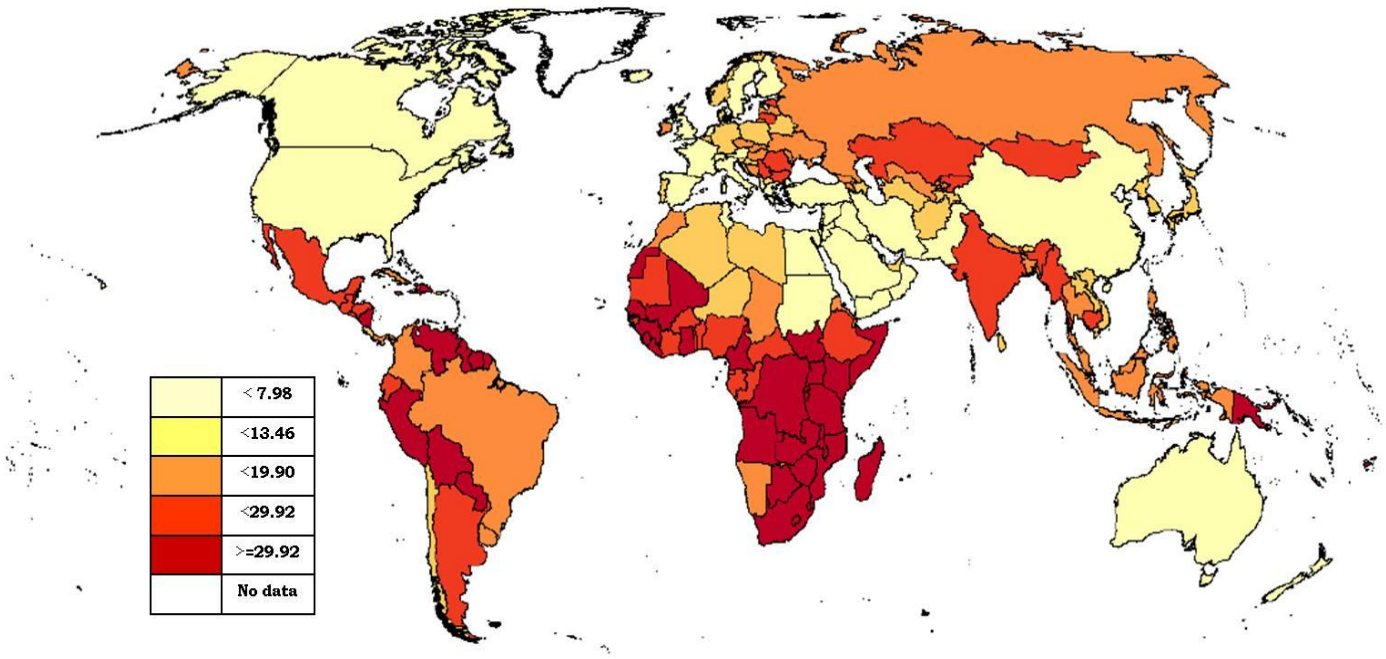
Fig. 4. The Global contribution of each HPV types to the burden of cervical cancer (Munoz *et al.,* 2004).



HPV Diagnosis Methods and Challenges

In spite of the opportunity offered by screening programs, cervical cancer remains the second most common cancer among women worldwide, with the estimated 4,93,000 new cases and 2,74,000 deaths in 2002. This is due to the fact that the majority of women in the world do not have access to the cervical screening, which can prevent up to 75% of cervical cancer. Cervical cancer clusters in developing countries where 80% of the cases occur, and it accounts for at least 15% of all female cancer. A different testing method whether it is analytical or clinical based plays an important role in early diagnosis (Table 2). The global burden of cervical cancer remains high in the world, notably in developing countries without screening programs.

Fig. 5. Age-standardized incidence rates of cervical cancer in World.



Data sources: Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. GLOBOCAN 2012 Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer; 2013.

Table 2. Characteristics of tests for the detection of cervical cancer and its precursors.

Test sensitivity/specificity for CIN 2/3 lesions and cervical cancer		
Test	Analytical	Clinical
Based on cell morphology		
Pap smear/tissues[a]	NA	low/high
Colposcopy[a]	NA	moderate/low
Visual inspection[a]	NA	low/low
Detection of HPV proteins		
Immunocito/histochemistry[b]	Low/High	Low/Low
Electron microscopy[b]	Low/High	low/low
Western blots[b]	low/high	low/moderate
Detection of HPV genomes		
Direct methods		
Southern blot [b,c]	moderate/high	moderate/high
In situ hybridization[b,c]	moderate/moderate	moderate/moderate
Dot blot	low/high	low/high
Signal amplification		
Hybrid Capture[d,e,f]	high/high	high/high
Target amplification		
PCR[d,e,f]	high/high	very high---high/high---moderate
Real-Time PCR[e,f]	very high/high	very high *
Detection of anti-HPV antibodies		
ELISA peptides	low/low	low/low
VLP	moderate/high	low/low
Fused E6/E7	high/moderate	low---moderate/high

a. Limited because of their low sensitivity; highly dependent on sampling and tissue preservation; cannot type HPV.
b. Technically cumbersome and/or time consuming.
c. Requires DNA and tissue preservation
d. Less dependent on sampling; can be done in crude samples
e. Suitable for high-throughput testing and automation.
f. Provides viral load information.

Table 3. Existing prophylactic vaccine properties a comparison.

	Cervarix	Gardasil
Clinical effectiveness	Prevention of high-grade CIN2/3 and cervical cancer causally related to HPV types 16 and 18 in girls/women aged 10-25 based on efficacy data in 15- to 25 years-old women, as well as bridging immunogenicity studies in 10 to 14 years-old girls and boys.	Prevention of high-grade CIN2/3, cervical, high grade VIN2/3, and genital warts casually related to HPV types 6/11/6 and 18 in girls/ women aged 9-26 years based on efficacy studies in women aged 15-26 as well as bridging studies demonstrating immunogenicity in girls and boys aged 9-15
Active ingredients	Each dose HPV 16 L1 protein (20mcg) HPV 18 L1 protein (20mcg)	Each dose: HPV l1 protein (20mcg), HPV 11L1 protein (40mcg), HPV 16 L1 protein (40mcg) HPV 18L1 Protein (20mcg)
Adjuvant	ASO4-monophosphoryl lipid A adsorbed on aluminium hydroxide	Alum- amorphous aluminium hydroxy-phosphate sulphate
Dosage and schedule	One 0.5 mL dose at 0, 1 and 6 months by IM injection in the deltoid region	One 0.5 mL dose at 0, 2 and 6 months by IM injection in the deltoid or anterolateral thigh

The etiology of the disease has been linked to persistent infection with a limited number of HPV types. Novel options for protection take advantage of the technology for HPV DNA identification and on the ability to use highly efficient vaccines against the most relevant of the HPV types.

Prophylactic HPV vaccine

The principle of prophylactic vaccines to prevent cervical cancer relies on the generation of neutralizing antibodies, which prevent subsequent infection with high-risk types of HPV. Virus like particles that structurally mimic the native virions can be produced by expressing the HPV type specific L1 proteins, using a recombinant yeast or baculoviral technologies. Currently, two licensed prophylactic vaccines have been developed and tested in large randomized trials. The first of these is a quadravalent vaccine directed against types 6, 11, 16 and 18, known as Gardasil, which is manufactured using yeast expression system and uses an aluminium adjuvant and is designed to prevent genital warts as well as cervical cancer. The second is a bivalent vaccine directed against types 16 and 18 which is known as Cervarix and incorporated a novel adjuvant, ASO4. Table 3 summarizes some key properties of the two vaccines.

Second Generation HPV Prophylactic Vaccines

The approved HPV vaccines, Gardasil® and Cervarix®, prevent infection by a subset of oncogenic HPV types, necessitating the development of highly multivalent L1 VLP vaccine or developing L2 as a single broadly protective antigen. It is estimated that HPV-16/18 vaccines will provide over 75% protection against the ICC in South Asia. HPV-45, -33, -35 and -58 accounts for an additional 20% of cervical cancer in this region. The addition of these additional HPV types in a second-generation vaccine could provide optimal cervical cancer prevention (Bhatla *et al.,* 2008).

Development of HPV-L2 Vaccine

Characterization and scale up of a type-independent, broad spectrum, safe and efficacious HPV vaccine based on the L2 protein, a key protein necessary for its infection.

A chimeric construct containing conserved nucleic acid regions coding for an L2 protein of HPV type 6B, 16, 18, 31 and 39 was designed. HPV types 16, 18, 31 and 39 are oncogenic and 6B causes genital warts. The chimeric construct when expressed in *Escherichia coli* produces a single fusion protein encoding the 11-88 amino acid (AA) residues of these 5 HPV types. The 11-88 AA region is involved in viral infection, is immunogenic, and is conserved across many types. The basic premise for developing this L2 based HPV vaccine is that the candidate vaccine will elicit broad cross-protective neutralizing antibody response against oncogenic and genital wart causing HPV types beyond those covered in this vaccine. The immunogenic regions represented in the fusion protein were selected based on the phylogeny and the clinical relevance. The production process was developed in recombinant bacteria (*E. coli*) and was devoid of animal or human derived raw materials. The purified protein was formulated with aluminum phosphate and delivered as preservative-free liquid formulation. The pharmaceutical form is a ready to use liquid vaccine formulation (0.5 ml per single human dose) in single dose glass vials.

Conclusion

Though the development of HPV prophylactic vaccines targeting the two important oncogenic HPV types, 16 and 18 is a major achievement for the biomedical researchers. Four-fifth of the cervical cancer burden is in the developing world where there is a lack of effective screening. It is a combination of tiered pricing, local manufacturing and new technology is required to realize the full potential of HPV vaccination worldwide. Several interesting and potential low cost candidates are in the phase of entering clinical trial shortly, including L1 calsomers, L2 polypeptide and *Salmonella typhi* expressing L1.

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