An overview of Human Papilloma Virus

A. A. D. Gayathri Upeksha Amarakoon\(^1,\*\), S. W. P. L. Daulagala\(^2\), Fara Fathima\(^2\)

\(^1\)Marine Biological Resources Division, National Aquatic Resources and Development Agency (NARA), No15, Crow Island, Colombo 15, Sri Lanka

\(^2\)Department of Microbiology, Faculty of Medicine, University of Peradeniya, Peradeniya, Sri Lanka

*E-mail address: upeksha@nara.ac.lk, upek_sha83@yahoo.com

ABSTRACT

Cervical cancer is the second commonest cause of cancer among women. According to the World Health Organization (WHO) data, in 2006 there were 1.4 million women who were suffering from cervical cancer and 80% of deaths among them were of women from developing countries. Genital infection with Human Papilloma Virus (HPV) is one of the major etiological factors for developing cervical cancers in almost all countries (World Health Organization, 2005). Papilloma viruses were first identified, cloned and sequenced from cervical tumor specimens and were subsequently established as important causative agents for the development of cervical cancer. Differences in prevalence rates are observed worldwide. Infection is more common in sexually active young women of 18-30 years and a reduction in acquisition of infection is observed after 30 years. The situation of Cervical cancer in Sri Lanka is identified as the second most common cancer among women in Sri Lanka and approximately 7.74 million women are included in the risk category. It is reported that nearly 850 die from cervical cancer each year.

Keywords: Papilloma virus; cervical cancer; RNA genome; control region
INTRODUCTION

HUMAN PAPILLOMA VIRUS

Papillomaviruses are small non-enveloped icosahedral viruses of approximately 50-60 nm in diameter. It contains a circular, double-stranded DNA genome (7000-8000 bp) that exists in a chromatinized state. 16 different genera are divided into four major levels known as alpha, beta, gamma and delta (according to a phylogenetic analysis among the sequences of 118 papillomavirus types).

The alpha genus contains the viruses associated with the development of mucosal tumors in humans. The beta genus is associated with the development of cutaneous tumors. HPV group of viruses include more than 80 different types associated with a variety of epidermal warts and skin lesions and some of which are associated with skin cancer. They primarily infect epithelia at either cutaneous or mucosal sites. Mucosotropic HPVs can be further subdivided into high and low risk types depending upon their degree of association with human malignancy. Low risk HPV subtypes (Type 6, 11) are associated with more benign skin lesions such as warts (papillomas). High-risk subtypes (Type 16, 18) can cause neoplasia (abnormal cell growth) or dysplasias (enlargement of an organ or tissue due to abnormal cell growth) and are associated with the development of cervical and anal cancers.

The HPV genome is divided into three main regions namely; the long control region (LCR), early (E) region and late (L) region. LCR regulates viral gene expression and replication. E region encodes proteins which requires for viral gene expression, replication and survival. It divides into 7 subgene products namely E1, E2, E4, E5, E6 and E7. L region encodes for structural protein secretion and they are known as L1 and L2. L1 (Major) and L2 (Minor) are structural proteins that are assembled late and spontaneously to form the icosahedral capsid.

E1 is the only enzyme encoded by the virus possessing DNA helicase activity. Once bound to the viral origin of replication and recruits the cellular DNA-replication machinery to drive viral DNA replication. E2 protein serves three major functions in the viral life cycle. The first is to regulate the expression levels of the other viral gene products. This depends on the binding sites occupied by the LCR to act as a transcriptional repressor or activator. Second, it recruits E1 to the viral origin and enhancing viral DNA replication. Third, it has a critical role in transferring the viral genome to daughter cells during division of the host cell. E4 is the most abundantly expressed viral protein. It has been linked to processes aiding viral DNA amplification and viral release.

E5 is one of the three oncoproteins encoded by the virus. Its mode of action is still unclear. It contributes quantitatively to the productive stage of the viral life cycle and has been closely linked with the regulation of growth-factor signaling pathways and immune avoidance. E6 is the second HPV-encoded oncoprotein. It cooperates with E7 to provide an environment suitable for viral DNA replication and to overcome cellular apoptotic processes. The most well characterized target of E6 from high-risk mucosotropic HPV types is the tumor suppressor protein p53 which is directed by E6 towards degradation. E7 is the third HPV-encoded oncoprotein. By targeting cell-cycle regulatory pathways controlled by the tumor-suppressor protein; pRb and the related proteins p107 and p130, it provides an environment favorable for the viral DNA replication by maintaining an S-phase like state in the differentiating keratinocytes.
When over expressed in various eukaryotic cells, L1 can self-assemble to form virus-like particles (VLPs). These VLPs are the basis for prophylactic vaccines against HPV, through induction of neutralizing antibodies. HPV infection occurs at the basal epithelium. Although the incidence of infection is high, most infections resolve spontaneously. A small proportion of infected persons become persistently infected and this is the most important risk factor for the development of cervical cancer.

**LIFE CYCLE OF THE VIRUS**

The life cycle of the virus is divided into four main steps which are entry, establishment of the non-productive infectious state, maintenance of the non-productive infectious state and
productive stage. HPV is specifically epitheliotropic and its life cycle takes place within stratified squamous epithelia.

**Subsequent steps in the life cycle of the virus:**

**Entry** - Entry is established that HPV initiate infection by penetrating through microtraumas in the epithelial to reach the basal cells which are believed to be the target cells for HPV infection. The mechanism for virus entry into the basal cells is not entirely understood.

**Establishment** - The non-productive infectious state is once HPV particle enters the host cell, the virus relies primarily on the host’s cellular machinery to replicate the virus DNA. The HPV genome becomes established as a low copy number nuclear plasmid inside in the infected basal cells. Within these cells only early viral gene products are expressed and consequently referred to as the non-productive stage of infection.

**Maintenance** - The non-productive infectious state is a hallmark of HPV infection. It is a prerequisite for the development of cancer. It requires the viral genome to be maintained over multiple cell divisions, but the mechanism is still unclear.

**Production** - It begins when the daughter cells derived from the infected basal cells. Virus starts to differentiate and delays the terminal differentiation programme of the cell. It redirects the cell’s DNA replicative capacity. Then it allows amplification of the viral genome and expression of the late viral genes which are necessary for the production of progeny virus and subsequent viral release.

![Figure 2. Life cycle](image-url)
CLINICAL MANIFESTATION

FACTORS CONTRIBUTING TO CERVICAL CANCER

There are several factors which contribute to facilitate HPV infection which thus leads to the formation of cancer. Some of those factors are tobacco smoking, parity fertility and oral contraceptive use. Immunosuppression and certain dietary deficiencies are other probable cofactors for the infection. Some of the studies suggested HPV infections are highly prevalent in almost every country and current evidence suggests that at least 50 percent of sexually active women are infected with one or more types of HPV. Although transmission is known to occur primarily through sexual contact, rates of acquisition and risk factors for infection are unknown.

The potential risk of infection from nonpenetrative sexual contact remains undetermined but there is a possible association between oral and penile contact and oral HPV which leads to oral cancer. HPV coinfection with HIV has also been identified as an established cofactor which increases the risk of cancer and HPV coinfection with \textit{Chlamydia trachomatis} and Herpes simplex virus-2. The biological factors are genetic, immunological host factors and viral factors (virus type, variants of type, viral load and viral integration). Even though these are important factors, the exact mechanism has not been clearly identified.

During an investigation carried out by “Wits Reproductive Health and HIV institute”, Johannesburg in South Africa with Sub Saharan African males, confirmed that many HPV infections in men are similar to HPV infections in women. In a small percentage of individuals, HPV infection tends to persist and later progress to genital warts, preneoplastic and malignant lesions of the anus, penis, oropharynx and recurrent respiratory papillomatosis. This study has demonstrated a high prevalence of HPV infection among men who have sex with men (MSM).

The high prevalence of HPV among MSM is associated with elevated anal cancer incidences which are estimated to be 44 times higher than that among the general population. HIV infected MSM are particularly at high risk of anal HPV infection (95%). Few studies have determined HPV prevalence at multiple anatomic anogenital sites in MSM population.

TYPES OF HPV INFECTION

\textbf{Anogenital warts:} Anogenital warts (AGWs) are the most common clinical manifestation of HPV infection. It is mainly caused with HPV 6 and 11 and highly infectious. 65% people whose sexual partner has genital warts are estimated to develop warts themselves. The estimated incubation period for HPV infection in order to develop genital wart is 2 weeks - 8 months but approximately 20-30% of genital warts will spontaneously regress or reoccur, resulting in significant psychological morbidity. Lack of circumcision and high number of HIV infection has been identified as risk factors for AGWs in men. A small study in Johannesburg among HIV-positive men with penile warts, confirmed that 85% were having HR-HPV as well. HPV-16 and 18 were most frequently detected among patients. These high rates of HR-HPV detection in men with HIV suggested that they are at significant risk for the future development of preneoplastic and neoplastic lesions which emphasize the importance of targeting, screening programmes for HIV-positive men with AGWs.
Cervical and Anal cancer: Cervical cancer begins in cells on the surface of the cervix. Over time, the cervical cancer can invade more deeply into the cervix and nearby tissues. Cervical cancer cells can spread by breaking away from the cervical tumor. They can travel through lymph vessels to nearby lymph nodes, liver or bones. The process of spreading of cancer cells from the tissue in which they arise to other tissues elsewhere is called metastasis. After spreading, cancer cells may attach to other tissues and grow to form new tumors that may damage those tissues.

The anal cancer in men appears to be increased when it is compared with women. Human behaviors like receptive anal intercourse and HIV infection are the most important risk factors for anal cancer. Anal cancer can be often seen in MSM, heterosexual men and HIV negative men among the general population.

The anal and cervical cancer incidents are high in HIV-positive women and MSM. Anal cancer should be noted that receptive anal intercourse is not a prerequisite for anal HPV infection or pre-cancer lesions or anal cancer. Piketty et al. demonstrated high rates of anal infection and squamous intraepithelial lesions in HIV-positive men with no previous history of anal intercourse. Anal cancer is considered to be biologically similar to cervical cancer.
It is preceded by a spectrum of intraepithelial changes and anal intraepithelial neoplasia (AIN) which can be graded similarly to cervical cancer. There is strong supportive evidence that high-grade AIN is a precursor to invasive cancer.

**Penile cancer:** A recently published report of HPV detection in cancerous and precancerous penile lesions of men demonstrated multiple HPV infections. The major risk factors for penile cancer are lack of Male Circumcision (MC), poor genital hygiene, AGWs and HIV infection. Though penile cancers are relatively rare in men HIV-positive men have an eight-fold increased risk of penile cancer which may be associated with higher HPV infection rates. Other risk factors for penile cancer that have been reported are smoking, early age of first sexual intercourse, high lifetime number of sexual partners, lack of condom use, chronic inflammatory conditions including balanitis and lichen sclerosus.

![Figure 5. Penile cancer.](image_url)

**Cancer of vagina:** Vaginal cancer is a less frequent cancer in women. The occurrence of vaginal cancer is limited and have analyzed only in fixed tissues for a few types of HPV-65. Vaginal tumors and their precursor lesions can be detected by using DNA detection method. According to phylogenetic analysis the most prevalent type of HPV found in vaginal cancer are HPV 66 and 67.

![Figure 6. Cancer of vagina.](image_url)
Head and neck squamous cell carcinomas: Head and neck cancer commonly refers to Squamous cell carcinomas (SCCs) arises in the upper aerodigestive tract (oral cavity, nasopharynx, hypopharynx and larynx). Most head and neck cancers are associated with tobacco and alcohol exposures and presented after the age of 60 years. More recently oropharyngeal SCC has been observed particularly in the palatine tonsils and base of the tongue. It occurs in younger age groups and in people who have never smoked. The proportion of oropharyngeal cancers is unknown. HIV-positive individuals appear to be at moderately increased risk of HPV associated head and neck SCC compared with the general population (D’ Souza et al.2008).

Adenocarcinoma of uterine cervix: The adenocarcinoma of cervix originates adjacent to the squamous epithelial neoplastic lesions. Though majority cases of cervical cancer are SCCs, Adenocarcinoma of cervix also contributes to the overall burden of cancer of uterine cervix. The higher proportion of adenocarcinoma cases has been observed in those areas where incidence of cervical cancer is low and in some western countries.

Cancer of vulva: The vulva cancer is not similar to cervical cancer. Distinct subtypes like the warty and basoloid types have been recognized but majority of tumors are squamous cell carcinoma. Etiologically vulva carcinomas are heterogeneous and thus the presence of HPV infection in invasive vulva cancer cases varies in subtypes 69 and 71. Vulva cancer with basaloid histopathology in young women is often associated with HPV-69. HR-HPV types 16, 31 and 33 are the frequently detected types in vulva cancer and its precursor lesions.
**Ovarian cancer:** Ovarian cancer is most commonly formed in the epithelial lining of the ovary resulting in epithelial ovarian cancer or in the egg cells resulting in a germ cell tumor. Ovarian cancer is the fifth leading cause of death due to cancer in women and the leading cause of death due to gynecological cancer. A woman has a lifetime risk of ovarian cancer of around 1.5%. The most number of cases represent malignant transformation of ovarian teratomas or, are associated with preexisting Brenner tumor or ovarian endometriosis. The actual cause of ovarian cancer is unknown. There is an increased risk of ovarian cancer in older women and in those who have a first or second degree relative with the disease. Some studies have demonstrated that HR-HPV types 16 and 18 play an important role in the development of ovarian cancer.

![Figure 10. Ovarian cancer](image)

**Cancer of urinary bladder:** Bladder cancers are transitional cell carcinomas of the epithelial cell lining of the bladder, ureters and urethra. Both genetic and environmental factors are responsible for causing this type of cancer. The most significant risk factor associated with bladder cancer is smoking. The carcinogens are absorbed through the lungs into the bloodstream and filtered out by the kidneys and enter the urinary tract. An environmental risk is presented by arylamines; people who are in the leather, rubber, printing and textile industries are exposed to these chemicals which are carcinogenic.

![Figure 11. Cancer of urinary bladder](image)
Nongenital warts: It is the most common warts on hands, fingers and knees. There are two types of warts which have been observed among the general population, known as planter warts and flat warts. Basically planter warts can be seen on feet and flat warts can be seen on hands and faces.

Figure 12. Laryngeal papillomas  Figure 13. Plantar warts

Cancer of eye: HPV 16 and 18 E6 gene expression was found in intraepithelial neoplasia of the conjunctiva. Since majority of retinoblastoma is sporadic, it is due to the inactivation of Rb gene, the product of which binds to HR-HPV E7 protein. HPV could be transmitted in utero during vaginal delivery or otherwise. Therefore, it is important to look for prevalence of HPV infection in retinoblastoma cases.

Figure 14. Conjunctival papillomas

Nasopharyngeal carcinoma (NPC): Nasopharyngeal carcinoma is an uncontrolled growth of cells that begins in the nasopharynx, the passageway at the back of the nose.
OTHER CANCERS

Cancer of lung: There are several reports on the association of HPV infection with lung cancer which are rare but also controversial. The formation of the lung cancer can occur due to HPV 18.

Cancer of Esophageus: Esophageal Squamous Cell Carcinoma (ESCC) is highly divergent and demonstrates wide regional variation in incidents reported. It occurs mainly due to the HPV serotypes 16 and 18. Agarwal et al. 1998 reported a significantly higher number of esophageal cancer cases immune positive for HPV16 E6 oncoprotein. The most important reasons for the infection are different food habits, smoking and tobacco chewing. Ethnicity showed a significant difference in the frequency of HPV infection.
DIAGNOSTIC METHODS AND RESULTS

Different diagnostic laboratories follow different procedures to detect HPV infection.

SAMPLE COLLECTION

Smears of exfoliated cells from the cervix are collected with a spatula and endocervical brush. The Ayre’s spatula and endocervical brush are then rinsed by vigorous stirring in 20 ml of Dulbecco’s phosphate buffered saline with 1% penicillin/streptomycin/Fungizone (DPBS/1% PSF) in sterile 50-ml screw-capped Falcon tubes to provide cells for HPV genotyping. Samples are stored in Styrofoam cooler boxes with frozen gel packs and transported to the laboratory within 8 hours of collection.

PAP (PAPANICOLAOU) SMEARS IDENTIFICATION

This diagnosis indicates cell abnormality but it is not sufficient for a definitive diagnosis of a squamous intraepithelial lesion. Women who have these abnormalities are referred to get a colposcopic biopsy for analysis. The invasive cervical cancer is one of the preventable types of cancer due to the effectiveness of the Pap test. Half of the women who develop cervical cancer have not had regular Pap tests. There are two main methods and one method involves brushing the cervix with a spatula and broom and then ‘smearing’ what is collected on a slide. The second and more commonly used method in the USA currently involves collecting a specimen with the spatula and broom and then putting it into a liquid that can be plated onto a slide.

Figure 17. Pap smear preparation.
HISTOLOGICAL IDENTIFICATION

The first and last sections of the particular tissues are stained using haemocytone and eosine (H & E). The slides are examined by a pathologist without the knowledge of HPV status for the presence of neoplastic cells, extension of necrosis, extent of keratinization and evidence for maltreatment.
Verruca vulgaris (common wart) is caused by various strains of human papilloma virus (HPV 1, 2, 4, 7, 26-29). Macroscopically it may present as hard, rough surfaced papule (2–20 mm) and microscopically it is an exophytic, symmetric, papillomatous lesion with large keratohyaline granules and characteristic inturning of the rete ridges. Parakeratotic columnar tiers of stratum corneum overlie the papillomatous surface. Small amounts of haemorrhage may be present within the columns of parakeratosis. Other characteristic features include koilocytosis, hypergranulosis and presence of multinucleated cells.

**HPV SEROLOGY**

Antibodies against HPV 16 VLP are detected using ELISA and antibodies to HPV, E6 and E7 proteins are measured with RIPA *Invitro* translated $^{35}$P labelled full length E6 and E7 proteins.

**Host immune response**

Most encounters of HPV are cleared by the host between 1-2 years. Strong immune responses are usually not generated. HPV can be a chronic infection. Skin warts generate low levels of antibodies. 50% of IgG found against HPV 6 and 11 genital serotypes. IgG and IgA humoral response to HPV 16 associated CIN found among the patient’s samples (50-75%). Antibodies detected in 15-25% of those without any current infection (most likely indicates past infection). Antibodies are rarely detected in patients with pre-malignant cervical lesions. Antibodies were detected in 50% of women with late-stage invasive HPV-16 associated cervical carcinoma. Cell mediated immunity (CMI) is probably extensively involved in the control of infection. High incidence of cutaneous and anogenital HPV associated disease is observed among patients with genetic or acquired CMI deficiencies. Immunological and genetic aspects are likely to attribute to the persistence and progression of CIN. Higher frequency of HPV can be found in HIV infected women.

![Figure 20. Immune response](image)
MOLECULAR RESEARCH TECHNIQUES

- **In-situ hybridization**: In-situ hybridization is the only method which permits direct visualization of virus in the morphology context. It applies to formalin-fixed paraffin-embedded tissues. It has a very sharp specificity, but cross-hybridization cannot be totally avoided. The results are very technique-dependent and integration status can be determined.

- **Dot blot**: Dot-blot hybridization method is used for HPV DNA specimen analysis. Therefore, it is called as filter hybridization. The genital swab sample and the oral sample are amplified in duplicate with the consensus primers MY09, MY11 and HMB01 and with human β-globin control primers. The products of these amplifications are then probed with a biotin-labeled generic probe designed to detect most genital HPV types. Specimens found positive by generic probe are tested, with individual and mixtures of biotin-labeled, type-specific oligonucleotide probes to determine the presence of HPV types 6, 11, 16, 18, 31, 45 and 56. Samples hybridizing with the generic probe but not with one of the type-specific probes are classified as positive for uncharacterized genital HPV types.

- **Polymerase chain reaction (PCR)**: β–Globin PCRs are performed using four primers with the combination of spanning 100, 209, 326 and 509 bp to assess the quality of the DNA (duplex PCR).

- **Southern blotting Analysis**: HPV positivity is assessed by southern blot analysis of PCR products with general probes of HPV specific (αP\textsuperscript{32}) dCTP labeled DNA fragment derived from cloned DNA of HPV 6, 11, 16, 18, 31 and 33 as described. PCR products from GP5+/6+ PCR positive specificity are typed by stringent southern blot analysis with (αP\textsuperscript{32}) dCTP random primed labeled full length cloned HPV DNA types 16, 18, 31 and 33.

**Sample processing for HPV genotyping**

The cell suspensions are centrifuged at 1,500 rpm for 7 minutes. The supernatant is discarded and the pellets are re-suspended in 1 ml of DPBS/1% PSF. Then divided into two 500 µl aliquots and stored at -70 °C until use in DNA extraction. Each 500 µl aliquot of the cervical cell suspension is diluted to a volume of 1 ml with phosphate buffered saline (pH 7.4). The extraction can be done by using the DNA extraction kits. Different kits give different volumes for the extraction steps.

**HPV amplification**

The presence of HPV DNA in cervical cell suspensions is determined by nested PCR. It has two main steps. In the first round, amplification of the L1 region of the HPV genome with the MY09/MY11 consensus primers which are followed by a second round of amplification of the first round products with GP5+/GP6+ primers. The GP6+ primer is modified at the 5' end by conjugation with biotin and the introduction of phosphothioate bonds between the last five nucleotides for Luminex detection.

PCR for HPV PCR is performed on extracted DNA using primers from consensus sequence, spanning the E1 open reading frame of the HPV genome to detect types 6, 11, 16, 18, 31 and 33.
Forward primer - 5’-TGCTAAAAACGTTGTGC-3’
Reverse primer - 5’-GAGCTGTGCTTAAATTGCTC-3’.

Positive samples thus obtained are subjected to type specific PCR for HPV types 16 and 18. PCR is performed using type specific primers for HPV 16.

The forward and reverse sequence of HPV type 18 specific primers are 5’ACTATGGCGCGCTTTGAGGA-3’ and 5’GGTTTCTGGCACCAGCAGGCA-3’ respectively. The generated fragments are of 109 bp and 334 bp for HPV 16 and 18. The detection has been done by using ELISA which is specific for HPV 16 and HPV 18 using VLPs in order to proceed for the L1 region of the HPV gene.

The first and second round PCR is performed in a 100 µl reaction volume using 2 µl of the first round reaction mixture and the same reagent concentrations as for the first round reaction. The cycling conditions are 94 °C for 5 minutes, followed by 30 cycles of 94 °C for 30 seconds, 40 °C for 20 seconds and 72 °C for 30 seconds with a final extension step being carried out at 72 °C for 7 minutes. Amplification of the β-globin gene is used to assess the quality of the DNA extracts, according to Goleski et al. in 2008. Six products are visualized through gel electrophoresis on a 2% agarose gel, stained with EtBr for both the HPV and the β globin PCRs.

Figure 21. Gel picture of samples which have been subjected to Agarose gel electrophoresis (Modified from Stone et al. 2008)
Consequence primer mediated PCRs

Consequence PCR primers GP5+/6+ and CP1/2 are used for amplification GP5+/6+. PCR is performed with biotinylated primer (bioGP6+) to enable subsequent typing enzyme immunoassay. There are several studies on HPV which have been carried out by different research institutes. According to those studies, molecular detection was the base for viral identification.

A new HPV-DNA test for cervical-cancer screening in developing regions

A new test (care HPV; QIAGEN, Gaithersburg, MD, USA) has been developed to detect 14 high-risk types of carcinogenic HPV. Women have been screened for CIN. A cross-sectional study was conducted to assess the clinical accuracy of care HPV as a rapid screening test in two county hospitals in rural China. Research duration was from May 10th to June 15th in 2007. The care HPV test was done locally by using self-obtained vaginal and provider-obtained cervical specimens from a screening population-based set of 2530 women aged between 30 to 54 years in China. All women were assessed by visual inspection with acetic acid (VIA).

EPIDEMIOLOGY, TREATMENTS AND PREVENTION

EPIDEMIOLOGY

HPV is the most frequently diagnosed sexually transmitted infection in the world and it’s recognized as a public health problem for its role as a critical factor in developing various types of cancers. Differences in prevalence rates are observed worldwide. Infection is more common in sexually active young women of 18-30 years and a reduction in acquisition of infection is observed after 30 years. Cervical cancer caused by HPV is the most common type and it’s preventable. Approximately 6.2 million new HPV infections occur every year in the United States and approximately 20 million individuals are currently infected. It develops following progression of uncleared HPV infection to high grade and eventually to invasive infection. Women with normal cervical cytology who are infected with high-risk HPV have an approximately 100 fold increased risk of developing CIN 3 compared with uninfected women. Screening can be directed at the target population for optimal utilization of resources. HPV are the principal cause of invasive cervical cancer and CIN. Health education, promotion of condom usage and the need to follow healthy hygienic practices are the most cost effective approaches in reducing the incidence of cervical carcinoma in resource-crunch societies. Molecular epidemiologic evidence clearly indicates that certain types were highly prevalent among women aged below 30 years with a peak in the 21-25 year age group and those who are above 30 years.

Transmission and risk factors for infection

HPV infections are transmitted mainly through direct skin to skin or mucosal to skin contact and prevalent in all sexually active populations. The virus is easily transmitted and
each genotype has its own characteristic tissue tropism and characteristic age specific peak transmission curve.

The number of sexual partners has been shown to be the main determinant of anogenital HPV infection in both women and men. The highest incidence of anogenital infection occurs in teenagers and young adults (Burchell et al., 2006). Circumcision and condom use have also been associated in reducing the risk of infection in men and their partners (Burchell et al., 2006; Dunne et al., 2006). It has been reported that smoking, use of oral contraceptives, parity, more pregnancies (more than three), not having had a Pap smear, other sexually transmitted agents, having three or more sexual partners, age at first sexual intercourse and host susceptibility may influence the risk of acquisition of HPV infection (Burchell et al., 2006; Moscicki et al., 2006).

A classification of HPV types based on their phylogenetic relationship has been proposed. These criteria should be based on molecular epidemiologic studies that provide risk estimates and on functional evidence of the oncogenic potential of the various HPV types. More than 80 HPV types have been identified and about 40 can infect the genital tract.

![Cumulative rate of HPV infection among college-aged women who were virgins at baseline](image1)

**Figure 22.** Cumulative rate of HPV infection among college-aged women who were virgins at baseline. Adapted from (Winer et al.)

![Seroprevalence of HPV 16 by age and gender](image2)

**Figure 23.** Seroprevalence of HPV 16 by age and gender (Modified from Stone et al. 2008)
The Centers for Disease Control and Prevention (CDC) estimates that at least half of all sexually active individuals will acquire HPV at some point in their lives. According to several studies at least 80% of women will acquire an HPV infection by the age of 50. In the United States, it has been estimated that 10% of the population have an active HPV infection, 4% have cytological abnormalities and 1% have infection causing genital warts. HPV 16 and HPV 18 are the most common types with a cumulative infection.

The vast majority of sexually active men and women have the possibility to get infected with HPV. The host immune system clears most of these infections. Some women will develop fulminant disease such as genital warts, cervical dysplasia and invasive cervical cancer. The preventive vaccines that lower the incidence of HPV infection and its associated diseases may offer a promising alternative to current therapies.

PERSISTENCY, LATENCY AND NATURAL HISTORY OF INFECTION

The estimated duration of the infection for individual types vary from study to study. Most HPV infections clear within 1–2 years. It has been reported that infections in older women last for longer and have greater risk of cancer (Castle et al., 2005).

One study showed the most persistent infections of those in which the same HPV type or group of HPV types are detected during two consecutive visits, but these two visits could be 4 months up to 5–7 years apart, leading to serious conceptual problems (Woodman et al., 2007). Persistence is insufficient for carcinogenicity because there are non-carcinogenic types like HPV 16. Persistent HPV infection should have prior conditions for the development of high-grade precancerous lesions in CIN and cervical cancer. Host susceptibility factors and immune responses are very important. CIN3 can develop in young women (Winer et al., 2005; Ault, 2007) within 2-3 years of infection. CIN3 lesions are very small and it takes few years for them to grow but the accuracy of the HPV infection can be determined by using nucleotide detection methods.

Behavioral determinants of HPV infection: Female monogamy is predominant but the key determinants of infection in women are the number of sexual partners, the age at which sexual intercourse was initiated and at least one sexual partner was an HPV carrier as estimated by his or her sexual behavioral patterns. HPV infection of men was investigated by using early epidemiological studies like questionnaires which is related to the sexual behavior of sexual partners of women, with and without cervical cancer. Female sex workers are the main reservoirs and transmitters of HPV infections. The probability is high in that carrier. The risk of developing cervical cancer has shown to be related to the presence of HPV on the penis or in the urethra of the sexual partner.

Follow up studies with virgins initiating sexual intercourse: A group of scientists have followed a research with the virgins who have not experienced sexual intercourse or not expected to harbor HPV on the cervix. HPV-positive specimens have been collected from the external genitalia of apparently virgin women.

HPV DNA prevalence in the genital tract to number of sexual partners in both sexes and a study about the women practicing prostitution: In all age groups HPV prevalence was the highest among female sex workers, followed by women who are attending STD clinics.
and who are incarcerated. Women from the general population had much lower age-specific HPV prevalence rates.

**HPV DNA on penis and cervical cancer in the spouse:** The identification of certain types of sexually transmitted HPV as agents etiologically linked to cervical cancer. The evidence for a role of men as carriers and vectors of oncogenic types of HPV emerged from studies that introduced HPV DNA detection in penile samples. The lack of association found between most variables related to male sexual behavior and risk of cervical cancer in high risk countries. HPV infection is widespread that it reduces the ability of case control studies to identify individuals at a higher risk. Cross-sectional HPV DNA detection in the penises of adult men is still poor.

**Male circumcision, penile HPV infection and cervical cancer:** A study on male circumcision and its association with cervical cancer compared the prevalence of HPV DNA in the penises of circumcised and uncircumcised men and estimated their wives’ risks of cervical cancer. Male circumcision also reduced both prevalence of genital HPV DNA and risk of cervical cancer in female partners, particularly and most strongly in women whose male partners had a promiscuous sexual history.

**OTHER ROUTES OF HPV TRANSMISSION**

The evidence for the nonsexual transmission of genital types of HPV has been reviewed by several researchers.

1. **Genital HPV infections (genital warts) may occur in infants, children and virgin adolescents and adults (Not sexually active).**

2. **Horizontal transmission of low-risk types of HPV:** Genital warts may transfer genital HPV not only to their sexual partners by genital-finger transmission but also horizontally to their children by touching them. Finger-conjunctiva transmission has been suggested by studies reporting the presence of HPV serotype 16 in squamous neoplasias of the human ocular surface and conjunctival carcinomas.

3. **Vertical and perinatal transmission of HPV from mother to child:** Few HPV infections detected in infants were probably caused by HPV contamination with horizontal transmission of low-level genital or nongenital HPV types. Perinatal HPV transmission is unequivocally demonstrated for recurrent laryngeal papillomatosis which is associated with HPV-6 and HPV-11. The risk for the juvenile form of laryngeal papillomatosis appears to be the highest in first-born infants delivered vaginally to adolescent mothers. Orogenital transmission is also possible.

4. **High-risk genital types of HPV have been detected in the mouth and oropharynx as well as the conjunctiva:** They have been associated with some cancers of the oral cavity and oropharynx and with conjunctival squamous cell carcinoma.

5. **Transmission via blood, breast milk and semen:** HPV has never been detected in blood. Transmission of HPV to infants via breast feeding has not been documented either. The possible role of semen as a source of transmission of HPV has been explored in several studies.
(6) Transplacental transmission: Several studies have shown some evidence of intrauterine HPV infection. In one of these studies, 24 of 37 samples of amniotic fluid from women harboring HPV DNA or with abnormal cytologic results were HPV positive by PCR. Another study detected HPV-16 DNA in cord blood specimens from neonates born to mothers who tested positive for HPV-16. Detection of HPV-6 DNA in infants born by cesarean section further suggests that prenatal HPV infection may occasionally occur, probably through ascending infection. Finally, a case report describing the detection of epidermodysplasia verruciformis (EV) related HPV types in amniotic fluid, placenta and cervical scrapings from patient with EV renders plausible prenatal transmission of EV-related HPV types.

OTHER ENVIRONMENTAL RISK FACTORS FOR CERVICAL CANCER

Long-term use of hormonal contraceptives: The evidence of an association between cervical cancer and the use of oral or other hormonal contraceptives is not entirely consistent.

High parity: Women who reported 7 or more full-term pregnancies and were HPV positive had a 4-fold increase in risk of cervical cancer compared with nulliparous HPV-positive women with similar characteristics. There was still a 2-fold increase in risk when women reporting 7 or more pregnancies were compared with HPV-positive women reporting 1 or 2 full-term pregnancies. It has been speculated that the general reduction in the mean number of births in developed countries over the last decades may have contributed to the reduction in cervical cancer incidence. The risk for women who have used OCs longer than 5 years and had more than 5 full-term pregnancies has 11-fold increased risk of cervical cancer.

Cigarette smoking: Smoking is associated with a significant 2-fold increase in risk of cervical cancer. These recent studies are providing growing evidence for a carcinogenic effect of cigarette smoking in women with persistent HPV infection and concluded that smoking was an independent risk factor for cervical cancer. The mechanisms by which cigarette smoking may affect cervical cancer (direct tobacco metabolites, indirect effects related to tobacco-induced immune suppression or reduced intake of dietary antioxidants) remain elusive.

Coinfection with HIV: The evidence of a possible interaction between HPV and HIV at the origin of cervical cancer was formally recognized. The subsequent literature largely confirmed the evidence, although confounders of the epidemiologic association tend to obscure the results. The patients with HIV is at high risk for cervical cancer compared with the long time needed between HPV infection and full-blown cervical cancer.

Coinfection with other sexually transmitted infectious agents: Sexually transmitted infectious agents have been repeatedly associated with cervical cancer. Nonspecific inflammatory changes have also been related to modest increases in risk for preneoplastic cervical lesions in HPV-positive women. The difficulty with the evaluation of such factors lies in the strong colinearity observed among all sexually transmitted infections and the limitations of some of the biomarkers currently used to assess ever exposure or persistent exposure.
TREATMENTS

- Genital warts can be treated by a doctor and by different methods. All treatment involves destroying the patient’s skin which has grown in a strange and annoying way.
- Podofilox gel: A patient-applied treatment for external genital warts.
- Imiquimod cream: A patient-applied treatment. Chemical treatments (including trichloracetic acid and podophyllin), which must be applied by a trained health care provider to destroy warts.
- Electrosurgery: Uses and electric current to burn off the warts.
- Interferon: An antiviral drug, which can be injected directly into warts
- Laser therapy: Lasers are simply very intense light sources. This light has an enormous amount of energy that heats the tissue enough that it vaporizes.
- Cryotherapy: Liquid nitrogen or cryotherapy is used to deep freeze the wart tissue. It uses liquid nitrogen which is applied to the wart, the water in the cells expands, thus exploding the infected tissue. The exploded cells can no longer hide the human papillomavirus from the body's immune system. The immune system then works to destroy the virus particles. Periungual area may scar if cryotherapy with liquid nitrogen is used improperly. Scarring could lead to permanent nail disfiguration.

![Cryotherapy to Treat Warts](image)

**Figure 24.** Cryotherapy

- Adhesive tape therapy: Place several layers of waterproof adhesive tape over the wart region. Tape doesn’t remove for 6-1/2 days. Then take off the tape and open the area to the air for 12 hours. Then reapply tape for another 6-1/2 days. The tape works best in the region around the fingernail, because the air tight, moist environment under the tape does not allow the virus to grow and reproduce.
- Salicylic acid therapy: Salicylic acid is stored either as a liquid to paint on the wart or as a plaster to be cut out and placed on the wart tissue. The area with the wart should be soaked in warm water for 5-10 minutes. The wart should then be pared down with a simple razor and then should be discarded. It doesn’t shave far enough to make the wart bleed. Apply the salicylic acid preparation to the wart tissue. It shouldn’t touch the other areas of the skin because of salicylic acid's potential to injure normal tissue.
Then directions on the package have to be followed accordingly. This would involve numbing the region around the wart and shaving the wart flat with the surface and light electrodessication of the base.

![Figure 25. Shave removal](image)

**PREVENTION OF HPV-ASSOCIATED INFECTION AND DISEASE IN WOMEN AND MEN**

Evidence of the benefit of several strategies to prevent HPV infection and subsequent disease in men has emerged in recent years. Studies have shown a greater protective effect of condoms in the prevention of HPV acquisition in men and women. The probability of clearing an oncogenic infection was 30% higher in men who consistently used condoms with non-steady partners. Consistent condom use has also been associated with the regression of penile lesions in men. Male Circumcision (MC) has been associated with reductions in the incidence, prevalence and persistence of HPV infection in men. MC has also been associated with a lower prevalence of flat penile lesions in men. A woman below 30 years of age should get regular Pap smears, if they are among the sexually activated population. The smoking habit should be decreased among the male and female population in order to prevent cancers and many other diseases.

The risk of the disease can be decreased by effective screening and vaccination. Before the introduction of screening and vaccination programs, the incidence of cervical cancer in many developed countries was similar to that in less developed countries. Rates of cervical cancer incidence and mortality have declined in the last 50 years in many developed countries. In less developed countries it is relatively stable. The absence of overall decline in less developed countries reflect the absence of screening and vaccination programs and the short coverage achieved when implemented. The impact of control measures in these countries will substantially reduce the global burden of cervical cancer. It is well known that well-organized cervical screening programs or widespread good quality cytology can reduce cervical cancer incidence and mortality. According to findings from different research studies, it has been shown that the need and utility of anal cancer screening programmes at present is
vital. Anal cancer screening programmes for men are likely to be controversial. Despite immune reconstitution associated with HAART. This therapy has a preventive effect on the development of anal cancer.

The introduction of HPV vaccination could effectively reduce the burden of cervical cancer in the future.

**HPV Vaccines**: Vaccines are the ideal form of primary prevention for infectious diseases and have been successful in the control of many other infectious diseases. HPV vaccines are very effective at preventing infection and disease related to the vaccine-specific genotypes in women with no evidence of past or current HPV infection. Protection lasts for at least 5 years. HPV vaccines will reduce the risk of cervical cancer. But it doesn’t eliminate the risk of a cancer. The screening programmes are very useful and more important interventions for cervical cancers. The principle of prophylactic vaccination relies on the generation of neutralizing antibodies against the high risk HPV types. Virus like particles (VLPs) that structurally mimic the native virions can be produced by incorporating the L-protein fraction of the virus.

Currently Two prophylactic vaccines have been developed and tested in large randomized trials. One is a tetravalent vaccine directed against HPV subtypes 6, 11, 16 and 18. This vaccine is designed to prevent both cervical cancer and genital warts. The bivalent vaccine acts against subtypes 16 and 18 and designed solely against prevention of cervical cancer. They are non-infectious because it does not have any biological materials. Recently scientists designed HPV vaccines against serotype HPV 16 and 18. Those are very aggressive serotypes. The tetravalent vaccine protects against low-risk genotypes 6 and 11.

A very small minority of women had already been infected with all four HPV vaccine types at baseline. It is not necessary to screen for HPV before vaccinating women. The vaccines have not yet been evaluated among patients with HIV, severe malnutrition and intercurrent malarial or helminth infection. The pap screening is not needed to decide on eligibility for vaccination and it will be needed among the vaccinated women. As an example these women will continue to be at risk of infection with other types of HPV that can cause CIN lesions and cervical cancer.

**Protection against infection and its clinical consequences**: The primary analyses were conducted among women vaccinated according to protocol who did not have evidence of past or current infection with the relevant HPV genotypes included in the vaccines until at least one month after the third dose. Both vaccines have demonstrated efficacy of over 90% against persistent infection due to genotypes 16 or 18 in women who received 3 doses of HPV vaccine. The detection of the vaccine has been done by using according to the phases trials. It is known as phase I trial and phase II trial.

**Cross-protection against other genotypes**: Both vaccines have shown some evidence of cross protection against HPV 31 and HPV 45. Those are closely related to HPV serotype 16 and 18. For the tetravalent vaccine, a study of ten vaccine recipients in the phase II trial who were seronegative and HPV DNA negative at baseline for HPV 6, 11, 16, 18, 31 and 45. Studies are continuing for both the vaccines.

**Duration of protection**: The vaccine is given in 3 doses at 0, 1 and 6 months, intramuscularly to the deltoid region. Antibody levels fall by about one log between the peak after the third dose and 18 months after vaccination and then level off. It remains high in the
blood. But the disease protection is not known. Early results from the tetravalent vaccine trials show an increase in antibody titers to a challenge dose given five years after initial vaccination. It has been demonstrated for up to 5 years post-enrollment in phase II studies.

The optimal target age group for HPV vaccines is pre-adolescent girls. It is recommended to females aged 9-16 years before sexual debut. The vaccination also has been recommended to females up to 26 years. It is recommended during lactation but should be avoided during pregnancy. The mechanism and duration of protection by HPV vaccines is cross protection. The cost and effectiveness of different strategies for vaccination and screening will improve predictions of the benefits of these new vaccines.

The potential future introduction of HPV vaccines creates opportunities for strengthening health systems by rapidly establishing new partnerships for vaccine delivery, financing and monitoring of impacts. There are several common and uncommon side effects of these vaccines. The uncommon side effects are gastrointestinal symptoms, pruritus, urticaria, arthralgia, bronchospasms and dizziness. These side effects differ according to the vaccine types. The vaccine has to be stored at 2-8 °C. It also can be given simultaneously with other vaccines. The vaccine has been licensed in many countries including Sri Lanka. There are a number of other key issues to be considered in the implementation of vaccination programmes. Those are the implications of the cervical screening in the presence of vaccination programmes, the importance of developing public confidence and getting a sufficient understanding of their purpose and the prospects for getting these vaccines into resource-poor countries where the vaccine is least affordable. The challenges need to be addressed, particularly the need to achieve primary prevention in countries with no means of secondary prevention.

**AWARENESS OF CERVICAL CANCER**

The estimation of HPV and cervical cancer cases is most likely an underestimation of the true incidence of the disease due to a low number of cervical screening facilities and lack of a national cancer registry. The limited availability of cervical screening, lack of accurate knowledge and awareness of cervical cancer is resulting for the increasing number of cervical cancer cases. Over 80% of the cervical cancer cases diagnosed at an advanced clinical stage which often have a very poor prognosis (Gyenwali et al., 2013). Women from both city and rural areas are found to have a low knowledge and awareness of cervical cancer, HPV and the HPV vaccine. Awareness of cervical cancer is positively associated with having knowledge in STIs, formal education on sexual behavior, current contraception use and having an abortion. Both men and women have been given the knowledge of STIs and formal education by using health literacy through awareness programmes (Dimmitt et al., 2013, Lam et al., 2013, Wang et al., 2014).

In a hospital based study, Gyunwali et al reported that illiterate women were 8 times more likely to be diagnosed with cervical cancer than literate women (Gyunwali et al., 2013). It is because many have inadequate access to health services (Haviland et al., 2014) exacerbated by poor transportation and perceived long wait times for treatments (Gyunwali et al., 2014). It potentially indicates poor potential of vaccine uptake. There are several programs which were targeting adolescent women by mentioning the name of the country and year (La Montagne et al., 2011). Another study suggests that women in the developing
countries who sought treatment of gynecological problems had higher levels of cervical cancer awareness. Health facilities in other developing countries are the major source of health information. It was found to be that doctors and the patients share knowledge about the cervical cancer during their clinical visits (Dhamija et al., 1993) which helps to spread awareness among the general population.

The situation of Cervical cancer in Sri Lanka is identified as the second most common cancer among women in Sri Lanka and approximately 7.74 million women are included in the risk category. It is reported that nearly 850 die from cervical cancer each year.

References


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