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Human Papilloma Virus associated Cancers: Diagnosis, Carcinogenesis, Epidemiology and Prevention

Sunil K Srivastava
Associate Professor, Department of Microbiology, Swami Shraddhanand College, University of Delhi, Alipur, Delhi, 110036, INDIA.

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Abstract
Human Papilloma Viruses (HPV) are small non-enveloped viruses that can integrate with the host genome or persist as an episome to cause chronic infections. These viruses are widely prevalent and transmitted by direct skin or sexual contact. They typically cause asymptomatic infections or benign growth called papilloma but can also cause malignancies like cervical cancer, anogenital, oropharyngeal, head and neck cancers etc. Of these, cervical cancer, is the third most frequently diagnosed cancer worldwide in women, but in India it is the number one. This paper reviews the underlying virology, genomic features, transmission, epidemiology, pathogenesis, associated malignancies, detection, and prophylactic measures like vaccines. These cancers, however, can be prevented by creating awareness, timely vaccination, early diagnosis and regular screening.
Keywords: HPV, Transmission, Cancers, Carcinogenesis, Detection, Screening, Vaccines

Introduction

Human Papilloma Viruses (HPV) constitute over two hundred genotypes of icosahedral non-enveloped, double stranded DNA viruses that upon infection present an array of disease symptoms ranging from lesions and benign warts on the epithelium to invasive carcinomas. Cancers caused by HPV include cervical cancer, anogenital, oropharyngeal; head and neck cancer (1). Cervical cancer globally is among the second most prevalent cancers, in women, worldwide (2) and the most common in India, where it constitutes the centrepiece for mortality in young women (3). According to the WHO, there are approximately 50,000 new cases registered annually with a 50% mortality rate (4) . The strong link between high-risk strains (HPV 16 and HPV 18) and cervical cancer by zur Hausen's lab spurred a revolution in Viral Oncology (5,6,7). This led to research by virologists from across the globe in epidemiology, viral transmission, pathogenesis, cancer mechanisms, drug targets and immune response to design antivirals and vaccines. Subsequently, randomized control trials were successful in evaluating the efficacy of prophylactic vaccines like: Cervarix – bivalent (8),Gardasil – quadrivalent (9) and later a nonvalent vaccine (10). The success stories of the vaccines developed for HPV demonstrate the translational potential of viral-host interaction studies.

Virology:

HPV belongs to the genera Papillomavirus within the family Papillomaviridae . Papillomaviruses have non-enveloped icosahedral capsids measuring around 55 nm and the viral genome consists of a circular double-stranded DNA molecule of approximately 8,000

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base pairs (11). There are over 200 known types of HPV(12). HPV are categorised in the alpha, beta, gamma, mu, and nu papillomavirus genera and include cutaneous and mucosal viruses (13). Different HPV types are referred to as genotypes and are classified based on the L1 gene DNA sequence. A distinct type has an L1 DNA sequence at least 10% different from any other HPV type (13). Mucosal HPVs are divided into high and low risk types based on their ability to cause cancer. There are 15 known high risk types -16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 ; 82 and low risk types include types 6 ; 11 (14). HPV is highly transmissible and its infection is very common (11,13). There are two major modes of acquisition: casual contact, as is seen with cutaneous warts, and sexual contact seen with the mucosal types affecting the vaginal and anogenital mucosa (15). HPVs are of particular significance to human health as they are the causative agents of a number of benign conditions, and more importantly, cervical, oropharyngeal; head and neck cancers.

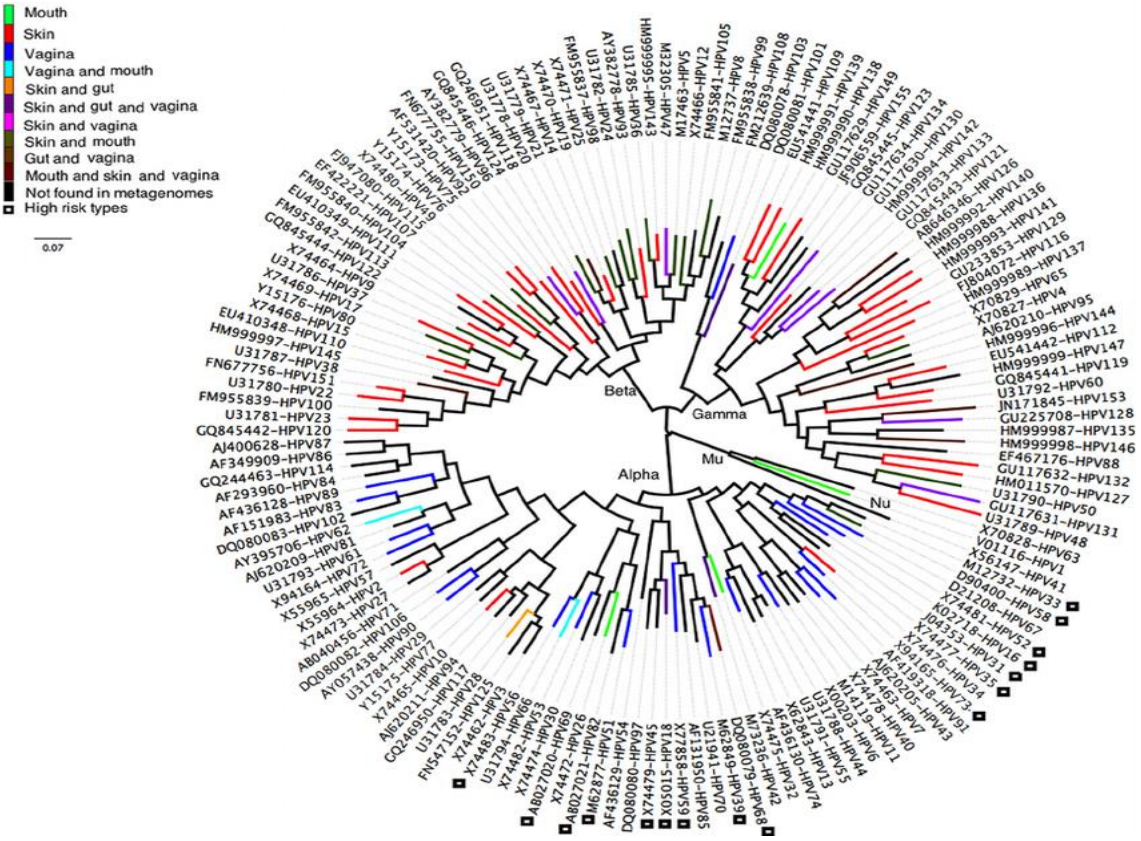

Fig 1 Genotypes of Human Papillomaviruses (Source HPV Nature Disease Primers)

Genome:

Human Papilloma virus genome is a circular double stranded DNA approximately 8 kb in size, with all the genes on one DNA strand. The virus genome is divided into three main regions: an early gene region (E), encoding various genes (E1-E7) that are expressed immediately after initial infection of a host cell, a non-coding long control region (LCR)and a late gene region (L) encoding the capsid genes L1 and L2 (13).

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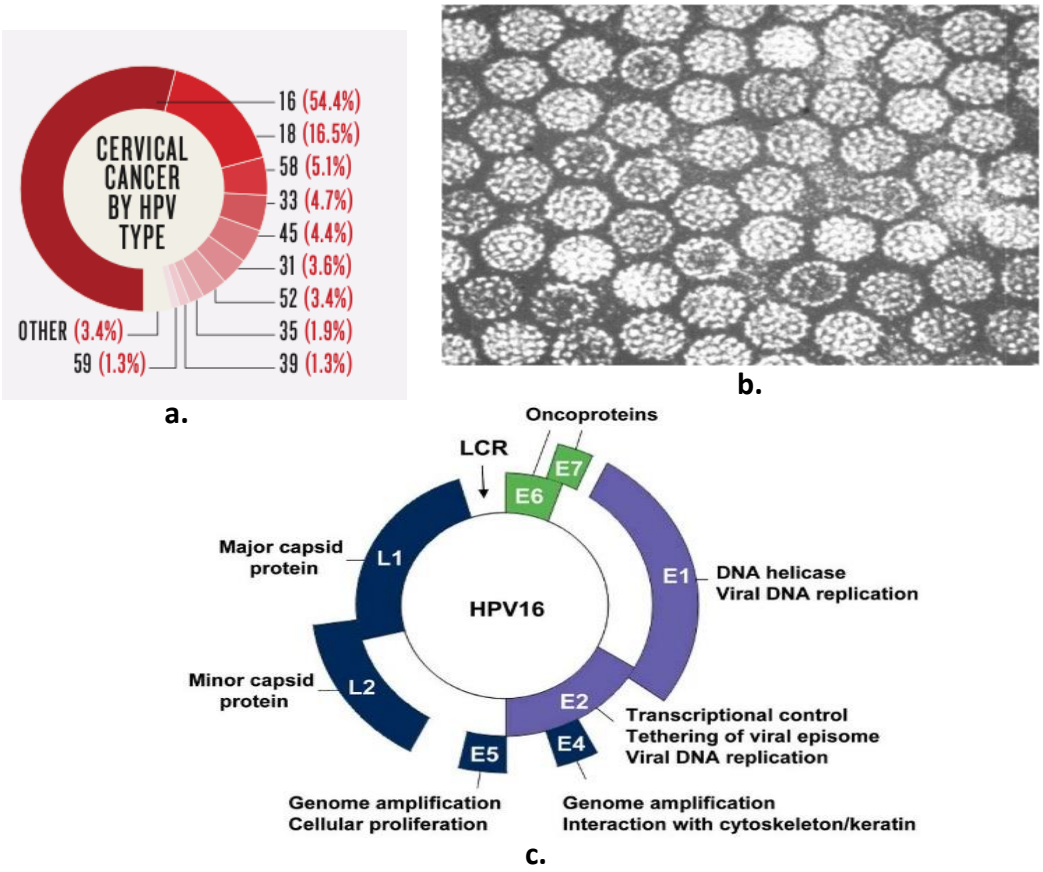

Fig 2 a. Strains of HPV implicated in cervical cancer, b. Electron Micrograph of negatively stained HPV, c. Gene organisation of HPV genome

DNA is required for malignant progression to occur (18). The major activity of the E6 protein is the inactivation of the tumour suppressor protein . Activated p53 acts to repair damaged DNA, arrest the cell cycle or induce apoptosis therefore preventing a build-up of mutations and genomic instability (17). In addition, the E6 protein has other transforming abilities including telomerase activation, blocking apoptosis, disrupting cellular adhesion, polarity, differentiation and the ability to reduce recognition by the hosts immune system (16,19).The E7 protein inactivates the retinoblastoma tumour suppressor protein (pRb) pathway through the breakdown of the E2F1-RB1 complex, resulting in the activation of E2F1 regulated S-Phase genes. The breakdown leads to aberrant S-phase entry and therefore uncontrolled cellular proliferation (20).

Transmission:

HPV is transmitted through close body contact and sex with an infected person. (21).

Cancers caused by HPV:

Various HPV types are responsible for a number of benign and malignant condition have been summarized in **Table 1**(21).

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Table 1: HPV related diseases and types associated

Disease	**HPV types associated**
Common warts	2, 7
Plantar warts	1, 4
Flat cutaneous warts	3, 10
Epidermodysplasia verruciformis	Over 15 types, including types 5 and 8
Focal Epithelial hyperplasia (oral)	13, 32
Oral papilloma's	6, 7, 11, 16, 32
Anogenital warts	6, 11, 42, 43, 44, 55 and others
Anogenital malignancies	16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66
Oropharyngeal cancer	16, 18 and anecdotal evidence for others

Laboratory Diagnosis of HPV:

Strategies for the detection of HPV are varied in their design and detection targets which may be HPV DNA or RNA, viral oncoproteins or other surrogate markers(38,39). HPV cannot be cultured in vitro and serological methods are ineffective as only around 50% of HPV infected individuals produce detectable antibodies (15).

The usefulness of any HPV detection strategy relies not only on the ability to detect the presence of HPV, but also to discriminate between a transient infection and an active infection with the potential for malignant transformation (39). Methods for the detection of HPV range from low sensitivity methods such as in situ hybridization, immunoperoxidase, and immunofluorescence (40) to moderately sensitive methods such as Southern blot, dot blot, reverse blot hybridization, and Hybrid Capture II (38,40).

Currently, the gold standard test is the detection of HPV E6/E7 mRNA as it reliably ascertains the presence of HPV and importantly, its biological relevance (39). However, this approach is limited in clinical practice because of the requirement of a fresh specimen, when most diagnostic pathology laboratories work with formalin fixed paraffin embedded (FFPE) biopsy samples (39). Polymerase chain reaction (PCR) detection of HPV DNA is the most sensitive method available (39).

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HPV related Cervical cancer Screening:

HPV awareness needs to be spread at all levels and screening camps organized from time to time especially in low and middle income countries. The presence of high risk strains of HPV have been commonly detected in many asymptomatic individuals which makes regular screening all the more important (41).Three screening modalities used for cervical cancer are discussed below.

1. PAP Smear Screening Diagnostic Test

Cytological technique is used to evaluate the structure of individual cells and their morphological abnormalities. Cervical cells are brushed or scraped from the cervix and stained with Papanicolaou, or Pap stain (42). There are two types of Pap smear. In the direct smear, collected material is smeared directly onto a slide. In Liquid based cytology collected material is placed in a solution containing fixatives in order to preserve morphology and separate out debris and non-relevant cells. The two most commonly used solutions are Thin Prep (contains alcohol) and Sure Path (contains alcohol and formaldehyde).

2. Visual Inspection with Acetic Acid Test

Vinegar is applied to the cervix. Normal tissue remains unaffected while neoplasms turns white. Lugol's Iodine can also be used which stains normal tissue brown. Although these tests have low sensitivity and specificity, they are inexpensive and rapid.

3. HPV Lab based DNA Test

In this test, a gene is selected, for which primers are designed. The DNA isolated from the sample is also tested for the presence of this selected gene by performing a PCR in the lab. The gene of which a probe is available can be detected in the sample by *In situ* hybridisation (ISH) and p16 immunohistochemistry.

4. **Biopsy**

If any of the above diagnostic methods indicate abnormalities then biopsy is done. Although the majority of cervical HPV infections resolve, some progress and cause precancerous lesions or cancer (18). Cervical precancerous lesions show abnormal cell growth in the basal epithelial layer. This precancerous state is categorized as Cervical Intraepithelial Neoplasia (CIN) and show a continuum of cellular changes. Histologically these show a range in severity from mild to severe dysplasia, CIN1 to CIN3.

Biopsy results can be interpreted at three levels:

- CIN 1 – Mild nuclear abnormalities, usually restricted to the lower one third of the epithelial layer.
- CIN-2 –Moderate number of cells with nuclear abnormalities usually restricted to the lower two thirds of the epithelial layer.
- CIN 3 ---Nuclear abnormalities span the entire depth of the epithelial layer.

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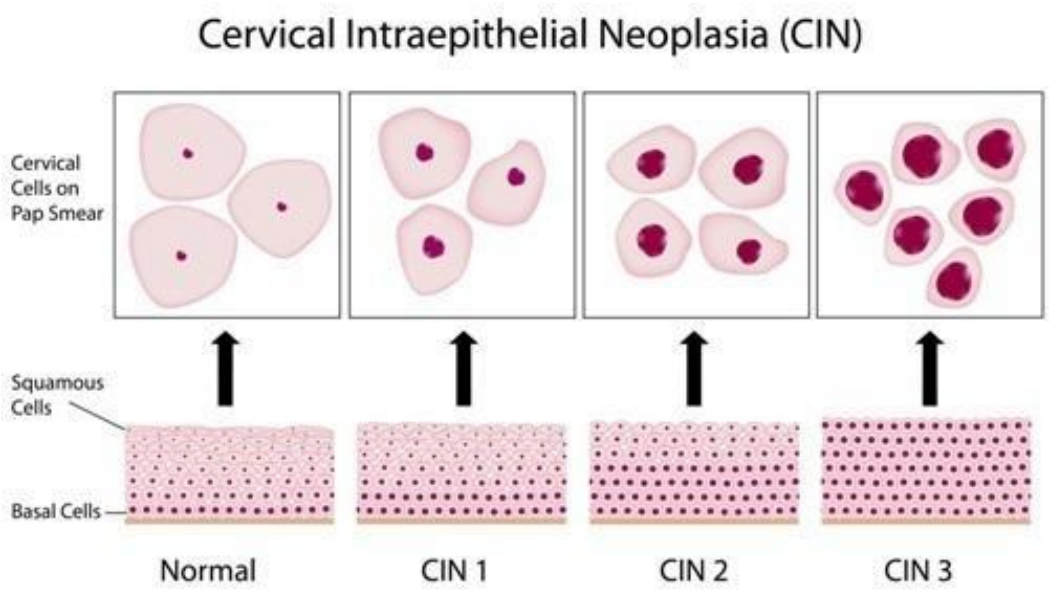

Fig 3 Stages of progression to cervical cancer. (Source: HP.com.tr)

Primary Prevention – Vaccination:

The knowledge that the HPV L1 protein could spontaneously assemble into virus like particles (VLPs), which mimic the natural virus and elicit protective neutralising antibodies, facilitated the development of prophylactic vaccines (44). Cervarix® and Gardasil® were initially the two vaccines approved for use in 2007 (8,9) and Gardasil®9 gained FDA approval in 2014 (10). Cervarix® is a bivalent vaccine for types 16 and 18, while Gardasil®4 contains types 16, 18, 6; 11 and Gardasil®9 has an additional five high risk HPV types -31, 33, 45, 52, and 58 (8,9,10,45) . All these variants of vaccines are also available in India; Gardasil (Sanofi Pasteur MSD/Merck) and Cervarix, (Glaxo Smith Kline). In addition, each vaccine has its own adjuvant used to promote immune response and enhance its durability. All these vaccines have almost 100 per cent efficacy. However, there is a need to reduce the cost and produce them indigenously.

Cervarix
Human Papillomavirus
Bivalent Vaccine

Gardasil
Human Papillomavirus
Quadrivalent Vaccine

Gardasil
Human Papillomavirus
Nonavalent Vaccine

Fig 4 HPV Vaccines currently available in the market

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