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Review Paper

Human papillomavirus and its oncogenic role in cervical cancers in sexually active women

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Abstract

Human Papillomavirsus (HPV) are ubiquitous in nature. At present, approximately 120 distinct genotypes have been identified in humans. HPV infections cause a variety of benign proliferations: warts (genital and cutaneous), cervical intraepithelial neoplasias, anogenital papillomas, oro-pharyngeal papillomas, and other types of hyperkeratoses. Cervical cancer is the most common cause of cancer-related death in women worldwide. Infection with high-risk HPV is established as the cause of cervical carcinoma; therefore, high risk HPV detection may have prognostic significance for the women who are at increased risk of disease progression. The lack of data on the incidence of cervical cancer and the prevalent strains in India makes it difficult to determine disease burden. Identification and accurate genotyping of the virus in cervical specimens is important to inform intervention policies for future management of HPV associated disease. This study also focuses on the various risk factors associated with persistence of HPV infection leading to cervical cancer. Describing the frequency and nature of HPV persistence, by HPV type, is important to understand its clinical significance and impact on clinical practice and management in the absence or presence of apparent cervical pre-cancer and cancer.

Keywords: Human papillomavirus, genotyping, cervical cancer, cervical intraepithelial neoplasia.

Introduction

Human Papillomaviruses (HPV) are members of the Papovaviridae family. They are small non enveloped virus containing double stranded DNA as their genetic material and are about 55 nm in size. It has an icosahedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 (major) and L2 (minor). The HPV genome is divided into 3 regions: i. Upstream Regulatory Region (URR), ii. "Early" region - which include the genes E1, E2, E3, E4, E5, E6, E7 and E8 and iii. "Late" region - which encodes the L1 and L2 structural proteins[1]. HPV is one of the most common causes of sexually transmitted disease in both men and women worldwide. *Papillomaviruses* are ubiquitous and have been detected in a wide variety of animals as well as in humans and are specific for their respective hosts. More than 200 types of HPV have been recognized on the basis of DNA sequence data showing genomic differences. Eighty five HPV genotypes are well characterized[2].

An additional 120 isolates are partially characterized potential new genotypes. HPVs can infect basal epithelial cells of the skin or inner lining of tissues and are categorized as cutaneous types or mucosal types. Cutaneous types of HPV are epidermitrophic and target the skin of the hands and feet. Mucosal types infect the lining of the mouth, throat, respiratory tract, or anogenital epithelium[3].

Since the 1840s, sexual activity had been suspected to be a risk factor for cervical cancer, and Harald zur Hausen reasoned that *papillomaviruses* might contribute to this cancer owing to their role in sexually transmitted genital warts[4]. It has been proved now that among women cervical cancer is one of the most common causes of death due to HPV[5]. However, percentage of women with anal HPV infections is found to be low in literature[6]. Only infection by HPV might not be sufficient to lead to cervical cancer, persistence of high risk HPV for a longer duration is also required that will lead to cervical intraepithelial neoplasia (CIN), a precancerous stage.

Epidemiology

Epidemiologic studies indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by a variety of factors. High-risk HPV infection is necessary but may not be sufficient for the development of cervical cancer. Cervical cancer depends on a variety of additional factors that are associated with cancer-associated HPV types[1].

Persistent high risk HPV infections are associated with CIN[7] that are graded into CIN1, CIN2 and CIN3, depending upon the severity of the lesion. However, other than persistent HPV infection, factors that increase the risk of HPV infection are: younger age, lower socioeconomic status, earlier sexual debut, multiple sexual partners, hormonal contraception, other sexually

transmitted infections, multiple childbirths, smoking, immunosuppression, and poor nutrition[8].

Minimum duration required for the persistence of approximately half of HPV infections is 6–12 months. The persistence of HPV infection could be defined as HPV positivity at two or more time points, not all required the same HPV type to be detected at consecutive visits (non-type-specific persistence) or the type specific persistence during the course of infection[9].

In a systematic review on the recurrence of new HPV infection even after completion of the treatment, have shown that the new HPV infections acts as the potential risk factor for the development of precancer and cervical cancer[10]. Thus, proving that a women once infected with the HPV infection is always more susceptible for getting new HPV infection throughout her lifetime if not screened properly at different intervals during her life time. However, noncompliance with cervical cancer screening and diagnostic programs is one of the major hurdles faced by the clinicians. The noncompliance of subjects could be because of the following reasons - especially with regard to transportation, child care, self-pay costs, education level and health care knowledge, or employment constraints[11].

Along with the various risk factors, reproductive factors were also found to be associated significantly higher with the incidence of HPV infection.[12] Although, more clear knowledge is still required to find the association between the two, so that strong preventive methods can be developed for the improvement of the strategies for the prevention and treatment of HPV-associated lesions. However a study on vertical transmission of HPV infection to the new born have shown that the absence of persistent HPV infection after 6 months of delivery, which shows that there is only probability of temporary inoculation of HPV DNA in new born if delivered through an infected cervix[13].

Faridi *et al*[14], in their study have mentioned that, in the coming year cervical cancer is going to be one of the most common cause of death especially in young women. In western countries its ratio is significantly high however this ratio varies throughout the world. According to WHO statistics, every year approximately 500,000 new cases are being registered out of which 250,000 are fatal. The most recent reports from USA showed that the women are more prone to this infection than the men. The overall reported percentage of getting infection in women irrespective of races was quite high being 17.9% while the chances of getting infection in men being comparably low as 8%[14].

International Agency for Research in Cancer (IARC) have shown that 5.17 per cent of all cancers, were attributed to HPV infection. The incidence rate (global) of cervical cancer was 16 per 100,000 women in 2002. There were estimated 493,000 new cases and 274,000 deaths due to cervical cancer in 2002 with more than 83 per cent cases occurring in developing countries[12].

In 2004, cervical cancer was the 5th most common cause of cancer death among women in the world, with 489,000 new cases reported and 268,000 deaths (3.6% out of 7.4 million cancer deaths)[15].

A population based survey in Bangladesh has concluded that even in Bangladeshi women, prevalence of HPV infection found to be similar to other regions of Asia. However, type- specific patterns were different[16]. Whereas, a study on prevalence and type distribution of high-risk HPV infection in genital warts of Korean men found to be significantly associated with the prevalence of high-risk HPV infection[17]. Although, the rate of anal cancer is higher among women than among men[6].

Indian scenario

Cancer of the cervix constitutes about 15-51 per cent of all female cancers and rates of age-standardized incidence range from 17.2 to 55 per 100,000 women in different regions of India. Infection of HPV 16/18 is estimated to account for more than 80 per cent of invasive cervical cancer including CIN3 and for 50 per cent of CIN2 lesions[18]. In India, 85-90 per cent cervical cancer cases are squamous cell carcinoma and the HPV 16 is the most prevalent type among them compared to other parts of the world where the proportion of HPV16 is much lower when both HPV16 and 18 are considered[15,18,19].

In India, HPV type 16 alone in cervical cancer is 70-90 per cent while occurrence of HPV type 18 varies from 3 to 20 per cent. Other high risk HPV types such as HPV 45, 33, 35, 52, 58, 59, and 73 have also been reported are rare and constitute only a minor group[20,21].

In a national HPV mapping study, prevalence of HPV type 16 was found to be highest in Chennai (88%), whereas it was very low in Jammu & Kashmir (14.2%)[22]. Interestingly, the peak of HPV infection, particularly HPV 16, appears to reach at later stage in third decade of sexual life at 26-35 years in Indian women in contrast to 18-25 years reported in western countries[23,24].

The role of HPV as a direct cause of cervical cancer has been shown in a study conducted at Banaras Hindu University; suggesting urgent need of screening programs and HPV vaccination in women with low socioeconomic status and those residing in rural areas[25].

Infections associated with HPV

***Papillomaviruses* in cancer of the cervix and in other anogenital cancers:** The mere presence of HPV DNA in the vast majority of biopsies from cancer of the cervix does not prove an etiological involvement; however, the presence of E6/E7 oncogenes is also required[3]. Different types of HPV found to be oncogenic and responsible for cervical worldwide[26]. Clinical manifestations of genital HPV can include genital warts

(condylomata acuminata), dysplasia and cancer of the cervix, anus, vulva, vagina and penis. Cervix being the most favorable place for the replication of HPV as there occurs a process known as squamous metaplasia, in which large number of transitional cells forms which supports the survival and replication of HPV[27].

***Papillomaviruses* in non-melanoma skin cancers:** Infection with cutaneous HPV (mainly the beta genus) seems to play a role in the development of non-melanoma skin cancers, especially squamous cell carcinoma[28]. However, the etiological association between non-melanoma skin cancers and HPV infection is difficult to prove as the same spectrum of HPV types is prevalent among healthy subjects[29,30].

***Papillomaviruses* in head and neck cancers:** The etiologic role of HPV in a large series of patients with head and neck squamous cell carcinomas (HNSCCs) has been shown in a retrospective study done at The Johns Hopkins University School of Medicine, Baltimore[31].

Oral and pharyngeal cancers are the sixth most common neoplasias in the world. Worldwide, the incidence of cancer at different head and neck sites varies by geographical region[32].

Genotypes of HPV: More than 150 genotypes have been identified and more than 40 are known to infect genital tract[33]. The original definition of specific HPV types as high risk viruses was based on their frequent presence in cervical and anogenital cancers. Durst M *et al*. and Pirisi L *et al*. have shown that high risk viruses were able to immortalize human keratinocytes whereas low risk viruses failed to do so. The ability of these *papillomaviruses* to code for mutagenic oncoproteins (E6/E7) seems to be the main factor for their characterization as "High-Risk" or "Low-Risk" viruses[3].

According to the classification of International Agency for Research on Cancer (IARC), based on available epidemiologic and mechanistic evidence of individual HPV carcinogenicity for cancer at any site, each HPV genotypes were categorized into more categories. Thus, 12 HPV genotypes (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59) were classified as "carcinogenic to humans" (Group 1), HPV-68 as "probably carcinogenic" (Group 2A) and 12 other HPV genotypes (26, 53, 66, 67, 68,70, 73, 82, etc) as "possibly carcinogenic"[33,34] being genotype 16 and 18 as the most carcinogenic. Low risk genotypes include HPV 6, 11, 30, 34, 40, 42, 43, 44, 53, 54, 55, 57, 61, 62, 66, 67, 69, 70, 82, 85 and 97[28] being genotype 6 and 11 most prevalent.

Pathogenesis: i. Transmission of HPV occurs primarily by skin-to-skin contact. ii. Basal cells of stratified squamous epithelium may be infected by HPV. iii. It is assumed that the HPV replication cycle begins with entry of the virus into the cells of the basal layer of the epithelium. iv. Once inside the host cell, HPV DNA replicates progress to the surface of the epithelium. In the basal layer, viral replication is considered to be non-productive, and the virus establishes itself as a low-copy- number episome by using the host DNA replication machinery to synthesize its DNA on average once per cell cycle. v. In the differentiated keratinocytes of the suprabasal layer of the epithelium, the virus switches to a rolling-circle mode of DNA replication, amplifies its DNA to high copy number, synthesizes capsid proteins, and causes viral assembly[1]. vi. It has been hypothesized that P53 and RB1 are highly vital tumor suppressor proteins and any change in their normal function will leads to the pathogenesis of several cancers. If there is any interaction between these two cellular tumor-suppressor proteins with E6 and E7 oncoproteins will lead to the inactivation of P53 and RB1 of HPV, has been widely hypothesized to play pivotal role in cervical carcinogenesis[35].

Virulence and oncogenes: Functionally high- risk HPV infection contributes to carcinogenesis and tumor progression predominantly through the actions of two viral oncogenes, E6 and E7[36]. Both of these oncogenes interact with and inhibit the activities of critical components of cell cycle regulatory systems, in particular E6 with p53 and E7 with Rb1 as shown in Figure-2[3,1].

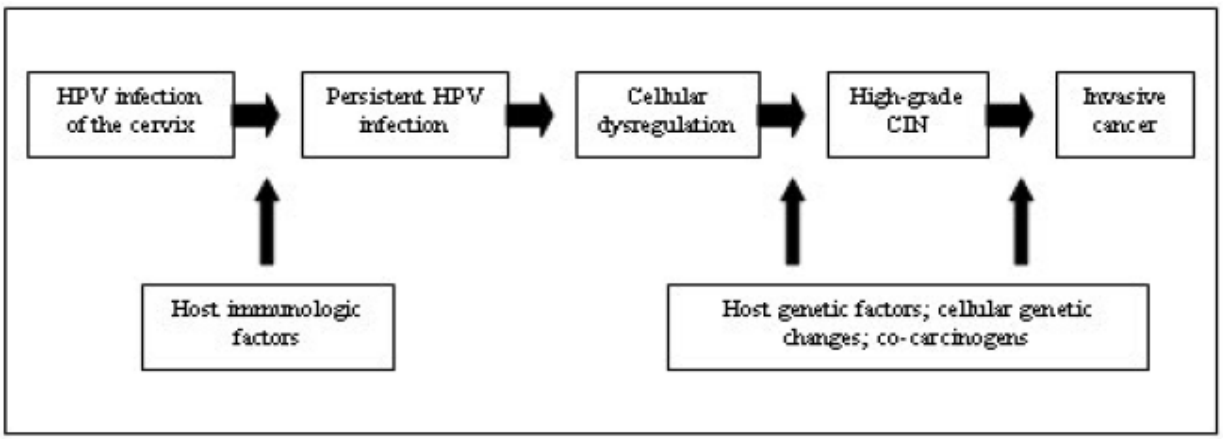
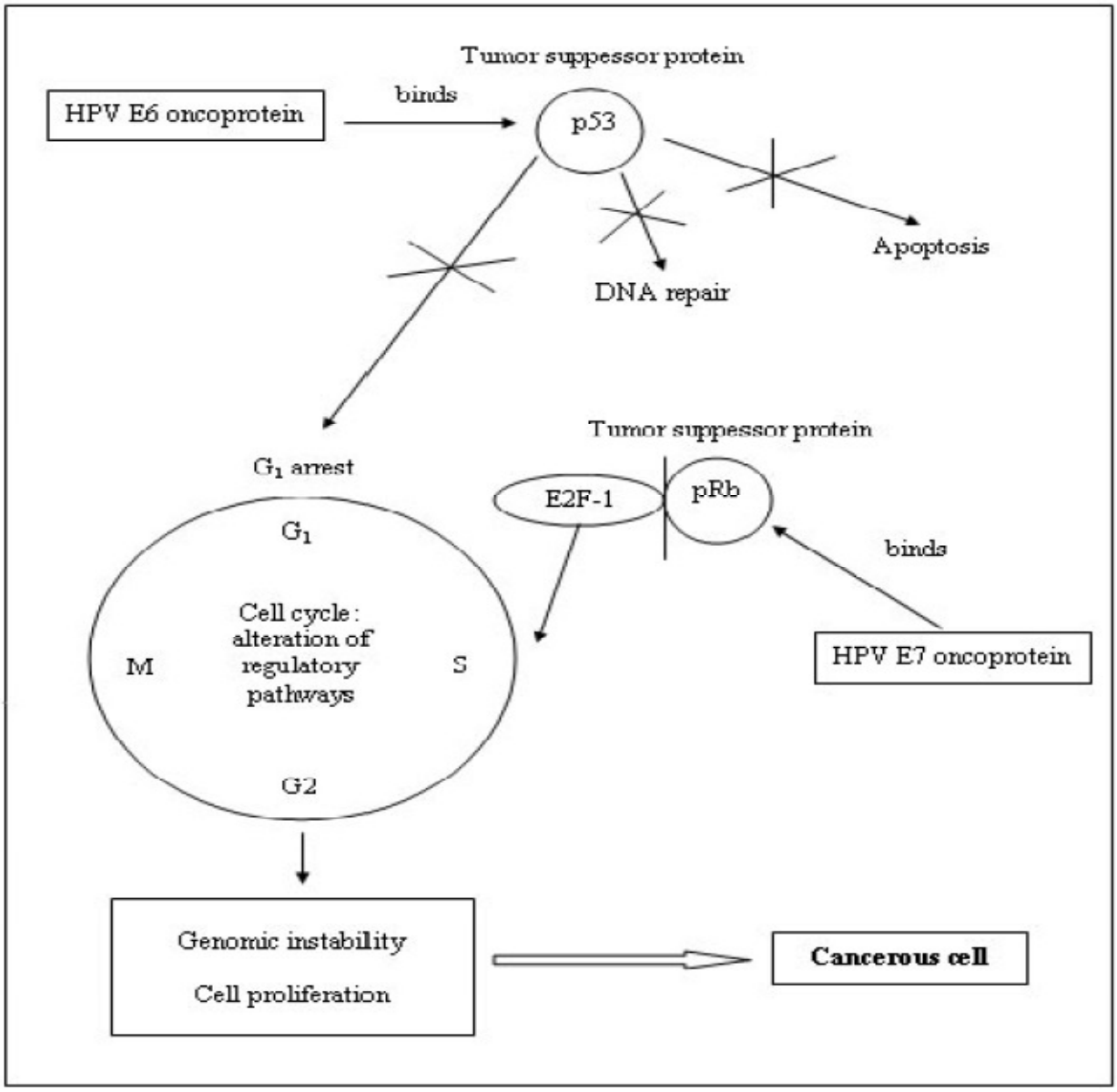


Figure-1: Developmental stages of cervical cancer.



. Molecular mechanisms of oncogenic HPV infection.

Figure-2: Molecular mechanism of oncogenic HPV infection.

Diagnosis

Histology: When a carcinogenic HPV type is detected by HPV testing, a concurrent cytological abnormality is present about most of the time. Histology provides the reference standard for cervical disease. At present, histology is based on morphology and does not consider HPV biomarkers. Advancing grades of CIN (grades 1–3) are distinguished mainly according to the amount of vertical extension of abnormal cells in the cervical epithelium. Abnormal cells restricted to the lower third are designated CIN1, abnormal cells restricted to the lower two-thirds are designated CIN2, and full- thickness extension of abnormal cells are designated CIN3. This division is arbitrary. Currently, a histologically confirmed CIN3 lesion is a clear indication for surgical treatment[37].

Morphologically it is difficult to differentiate high-grade lesions that will regress or persist from those which will definitely progress to invasive cancers. HPV have the ability to integrate their genomes into host cell chromosomes and transcribed into mRNAs encompassing viral and cellular sequences as found in most of the cervical carcinomas however in contrast in early preneoplastic lesions, HPV genomes persist as episomes. Therefore detection of HPV transcripts derived from integrated HPV genomes may specifically indicate both CIN lesions at high risk for progression as well as invasive cervical cancers[38].

Self collected sample vs. Physician collected sample: DNA methylation analysis as a triage test in high risk-HPV positive women could be an attractive alternative to cytology because it is feasible for direct brush-based self-samplers and showed good correlation with matched physician-taken samples[39]. This association between physician and self-collected samples was demonstrated by Ana P. Ortiz *et al.*[40] from Puerto Rico in 2013. They have also concluded that this method could be used as an HPV screening method among women[40]. However, a study on the detection of HPV-DNA using 3 different collection methods i.e. clinician-collected cervical specimens, clinician-collected cervicovaginal specimens, and self-collected cervicovaginal specimens taken at home and showed no significant differences between clinician-collected and self-collected cervicovaginal specimens ($P > 0.01$ for all comparisons)[41].

Real-Time Multiplex PCR assay vs. two versions (standard and modified) of Linear Array HPV Genotyping PCR assay: Molecular detection methods like, multiplex HPV PCR assays have been proved to detect more positive specimens than both s-LA and m-LA when clinical specimen DNA was isolated[42].

Rapid test: Johannes Schweizer et al. from Arbor Vita Corporation have used an immune chromatographic method for the detection of E6/E7 oncoproteins from HR-HPV 16/18 /45 and HPV E6 test prototype proved to be very specific for E6 oncoprotein of the targeted HPV genotypes.[43] However this feasible test kit is not available in many parts of the world, including India.

Prevention

Targeted counseling in heterosexual couples appears to reduce the number of infected sites and to speed up viral clearance[44]. However, a study conducted in Germany on the probability of acquiring HPV infection is more in women who were not prior vaccinated at younger age[45].

Currently, two successful prophylactic HPV vaccines-quadrivalent 'Gardasil' (HPV 16/18/6/11) developed by Merck while bivalent 'Cervarix' (HPV 16/18) by Glaxo SmithKline (GSK) are recommended for vaccinating young adolescent girls at or before onset of puberty. In these vaccines viral capsid proteins are present in the form of spontaneously reassembled virus-like-particles (VLPs) expressed either in yeast for 'Gardasil' or in baculovirus for 'Cervarix'. These two vaccines protect from infection with two of the most common cancer-causing HPV types 16 and 18 and more than 70% of cervical cancer cases are associated with these two HPV types. Both the vaccines were found to be highly immunogenic, safe, well tolerated and effective in preventing incident and persistent HPV infections including developing precancerous lesions[46].

However, a second generation vaccine is also been developing. The main goal of the second-generation vaccine is to develop vaccines that will be more suitable to resource limited countries: to reduce the cost of production, to have a longer shelf-life, single dose delivery, long lasting immunity, should be stable at room temperature and it could incorporate other oncogenic HPVs[47].

For invasive cervical cancers and other HPV-associated cancers, several treatment options are available including surgery, radiation therapy, and chemotherapy, alone or in combination depending on stage of disease. For cervical cancer, depending on the stage of disease at diagnosis, a woman may have the option to preserve her fertility or keep her ovaries. The survival rate five years after diagnosis of cervical cancer varies depending upon the stage of cervical cancer. The risk of survival decreases with higher stages of disease[48].

Conclusion

Lauren E. Wilson, while studying the natural immune response against eight oncogenic HPVs in a Triage Study found out that not all women infected with HPV develop antibodies to the virus, and the factors that may lead to this seroconversion have not been well characterized. In addition, there is currently no standardized assay for detecting HPV antibodies. In this study, the authors used a Luminex-based serology assay to measure antibody responses against eight carcinogenic HPV types. In women with mildly abnormal cervical cytology results, the study found an association between HPV-positive antibody titers and several risk factors for HPV infection. Smoking, however, showed a negative association with seroconversion[49].

An age specific global review on HPV antibody and DNA prevalence showed that females within the HPV vaccine eligible age group (9-26 years) had a range of dual HPV 16 DNA and serology negativity from 81-87%, whereas 90-98% was HPV 16 DNA negative. Serology and DNA data are lacking worldwide for females younger than age 15 years, the prime target group for vaccination[50,46].

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