

Review Article

Human papillomavirus infections

人類乳頭瘤病毒感染

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Human papillomavirus (HPV) infections are associated with a variety of benign and malignant conditions of the skin and mucous membranes. Of major clinical and public health significance is its relationship with cervical carcinoma. The pathogenetic mechanisms for HPV infection have gradually been elucidated, and the sequence of events in the initiation of carcinogenesis has been established. Laboratory diagnosis of HPV is mainly focused on the use of molecular techniques in cervical specimens for detection of HPV genotypes strongly associated with cervical carcinoma. Various treatment modalities for HPV infections are available, although none is distinctly superior. Prevention strategies are traditionally targeted on avoidance of infection via contact. Recent advances in vaccine development are promising in the prospect of eventual reduction of the disease burden of HPV infection.

人類乳頭瘤病毒(HPV)感染與多種皮膚及粘膜的良性及惡性疾病有關。其中極具臨床及公眾健康重要性的是此病毒與子宮頸癌的關係。HPV感染的發病原理正逐步受到瞭解，病毒致癌的各階段亦均相繼被確立。HPV的實驗室診斷主要是利用分子生物技術於子宮頸細胞樣本，以檢測與子宮頸癌有尤其密切關係的HPV基因型。治療HPV感染的方法有多種，但沒有一種特別有效。傳統的預防方法是避免從接觸受到感染。新近研制的疫苗將可能有效地控制HPV感染引致的疾病。

Keywords: Hong Kong, human papillomavirus infection, review

關鍵詞：香港，人類乳頭瘤病毒感染，綜述

Introduction

Human papillomavirus (HPV) is a DNA virus recently reclassified as a member of the

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family Papillomaviridae.¹ Over 100 genotypes of HPV have been identified so far.² The range of infections associated with HPV includes benign and malignant lesions of the skin and mucous membranes. Different HPV genotypes have different disease manifestations. Since the most important public health aspect of HPV infection is its association with cervical malignancies, most attention has been targeted at genital HPV infections.

Epidemiology

HPV infection occurs worldwide. Different age groups are infected by different genotypes with diverse presentations. The infection is transmitted via an abrasion in the skin or mucous membrane. Possible modes of acquisition include skin infection via autoinoculation and contact of bare feet with contaminated wet floor of public swimming pools and bathrooms, and genital infection during sexual intercourse. Laryngeal infection is probably acquired neonatally through the birth canal during parturition since the HPV types in the genital and respiratory tracts are similar. In Hong Kong, statistics from the Social Hygiene Service of the Department of Health showed that genital warts are the third most common sexually transmitted disease, after non-gonococcal urethritis/non-specific genital infection in males/females respectively, and gonorrhoea. Over 3,000 cases of genital warts are seen each year at the Service.³ Various studies using different laboratory techniques have been performed on the prevalence of HPV infection in different settings in Hong Kong. The figures ranged from 0% to 7.3% on routine endocervical examination in the general community setting,⁴⁻⁶ 12.6% to 30.6% in females attending social hygiene clinics,^{7,8} and 10% to 18.6% among those with normal or inflamed Pap smears in the colposcopy clinic

setting.^{5,9} The local prevalence is comparable with a worldwide average of 15% HPV detection rate among the general female population.¹⁰ Caution is required in interpretation of HPV prevalence data since diverse methods with variable sensitivity and specificity have been used.

Clinical features

The incubation period of HPV infection is usually two to three months, up to two years. The clinical presentations and their related HPV genotypes are shown in the Table 1. Infectivity probably lasts as long as visible lesions persist. The natural history is that lesions would regress spontaneously, usually synchronously, within months to years.

Skin lesions

A variety of presentations are encountered.¹¹ Plantar warts are painful, deep, endophytic warts found on highly keratinized areas of the hands and feet, most commonly in adolescents and young adults. Common warts are circumscribed hyperkeratotic, rough-textured, painless papules, varying in size from pinhead to large masses seen on prominent regions subject to abrasion such as hands and feet. They are found at less keratinized areas than plantar warts. Flat warts, also called plane warts, are smooth, slightly elevated, usually

Table 1. Clinical presentations of HPV infection and the related genotypes (genotypes in brackets are less commonly encountered).¹¹

Site	Clinical presentation	Genotypes
Skin	Plantar wart	1 (2, 4)
	Common wart	2, 4
	Flat wart	3, 10 (28, 41)
	Epidermodysplasia verruciformis	5, 8 (9, 12, 14, 15, 17, 19-25, 36, 46, 47) Malignant types: 5, 8, 17, 20, 47
Respiratory tract	Laryngeal papillomas	6, 11
Mouth	Focal epithelial hyperplasia	13, 32
	Oral papillomas	6, 11 (7, 16, 32)
Genital tract	Condyloma acuminatum	6, 11 (42, 43, 44, 55)
	Anogenital malignancies	16, 18 (31, 33, 35, 39, 45, 51, 52, 56)

multiple lesions varying in size on the face of youngsters. Epidermo-dysplasia verruciformis (EV) is an autosomal recessive hereditary cell-mediated immunodeficiency disorder which presents initially as flat warts in apparently normal children. Reddish-brown macular scaly patches usually appear in the first decade of life on the torso and upper extremities, caused by up to 20 rare HPV genotypes found almost exclusively in EV patients, presumably causing sub-clinical infections in normal people. Squamous cell carcinoma occurs in one-third of these patients within 10 years of clinical onset of EV arising from one or often several of the macular lesions at areas of skin exposed to sunlight, which is apparently a critical cofactor.¹² These lesions are usually associated with HPV genotypes 5 and 8. They are initially slow-growing in situ carcinomas, but may become invasive and metastasize.

Respiratory and oral lesions

Laryngeal papillomas are found on the vocal cords and epiglottis of young children and sometimes in young adults. Malignancy was associated mainly with irradiation in olden days. Focal epithelial hyperplasia occurs on the lip and is mainly described in Eskimos and South and Central American Indians.

Genital lesions

Most genital HPV infections are asymptomatic, subclinical or unrecognized. Visible genital warts, condylomata acuminata, are usually caused by HPV types 6 and 11. The lesions are cauliflower-like, fleshy growths most often seen in moist areas around the genitalia in both males and females. Flat condylomata of the cervix are lesions with dysplasia also known as low-grade squamous intraepithelial lesions (LSIL), previously cervical intraepithelial neoplasia type I (CIN I). These lesions may evolve into high-grade squamous intraepithelial lesions (HSIL) and invasive carcinoma. HPV types associated with the highest risk of genital malignancies are types 16 and 18, accounting for 40-60% and 10-20% of all cervical carcinomas respectively.² Other malignancies of

the genitalia, such as squamous cell carcinoma of the anal canal, vulva and penis are also associated with HPV. In Hong Kong, the most prevalent HPV types associated with cervical neoplasia are HPV 16 and 58, followed by other types, including types 18, 31 and 33.^{6,9} The high local prevalence of HPV type 58 is not seen in other Western countries. The implications for this finding are related to the need for diagnostic assays to be able to detect this HPV type, and to target this type when considering vaccine development.

Pathogenesis

HPV has greatly restricted tissue tropism, replication occurring only in the epithelial cells, keratinocytes, of the skin or certain mucous membranes. Different HPV types display preferences for different body sites. In general, infection occurs when breaks in the skin and mucosal membranes enable HPV to access basal keratinocytes. The virus gradually spreads to adjacent cells over weeks to months. Expression of viral genes stimulates proliferation of the basal cells and aberrant differentiation of cornified keratinocytes, causing warty lesions and LSIL in the cervix. Regression of lesions is mainly mediated by cellular immunity.^{2,11} HPV genomes may persist for years, and integration of viral DNA may occur, especially for high risk HPV types. Viral DNA is regularly found in the nuclei of premalignant cells in cervical dysplasia. In the cervix, HSIL may develop from LSIL or arise spontaneously. HSIL is the most predictable precursor of cancerous lesions with 30% or more of such lesions invading the basement membrane if untreated. HPV as the cause of cervical carcinoma is now generally accepted.¹³ Integration of the HPV genome into host cells often results in inactivation of the E2 viral repressor protein, leading to overexpression of the viral oncogenes E6 and E7 of high-risk HPV types. E6 and E7 proteins have been shown to bind to and inactivate normal cellular tumour suppressor gene products p53 and pRB

respectively. Normal cell cycle control and DNA repair mechanisms are affected, resulting in sustained cell growth, culminating in malignancy.² Other reported co-factors for cervical carcinogenesis include immunosuppressive conditions such as AIDS, use of oral contraceptives and cigarette smoking.¹³

Diagnosis

The diagnosis of HPV infection is usually evident clinically with the typical lesions. Endoscopy such as colposcopy and laryngoscopy may be required to visualize mucosal lesions. The Pap smear is used for detecting cellular abnormalities, both benign and neoplastic, in the cervix. Cytologic changes in HPV infection include abnormal keratinization, nuclear atypia and vacuole formation. In case of diagnostic uncertainty and suspicion of malignancy, excision and histology examination may be required. Microscopic features suggestive of HPV infection include thickenings, or acanthosis, of the stratum spinosum and stratum corneum. Presence of abnormal cells, koilocytes, in the stratum granulosum with irregular nuclei and large cytoplasmic vacuoles is the hallmark of HPV infection.

Virological investigation is usually not necessary for diagnosis of HPV infection. Recently, HPV detection followed by genotyping has been used as a means of identifying patients harbouring HPV types with high malignancy potential. The major application is to triage cases with a Pap smear result of atypical squamous cells of undetermined significance (ASCUS). Recent guidelines recommended reflex HPV DNA testing on liquid-based cervical cytology specimens and referral of cases positive for high-risk HPV types for colposcopy and directed biopsy.¹⁴ This strategy is preferred over two other approaches. The first involves interval repeat cervical cytological testing, which necessitates two examinations at 4- to 6-month intervals, with the disadvantage of prolonged

patient anxiety. The second approach is immediate colposcopy, which would increase use of healthcare resources.

Regarding laboratory techniques for HPV detection, the only practical method is nucleic acid detection, since the virus is not cultivable in conventional cell culture systems. Reported techniques include nucleic acid probe methods and nucleic acid amplification using the polymerase chain reaction (PCR).¹⁰ The Hybrid Capture Test is a commercially-available method using RNA probe mixtures to detect HPV DNA of high or low risk types. This is a sensitive test for screening for HPV infection. Cross-hybridization leading to false positive results has however been reported.¹⁵ The consensus PCR assay is widely used and targets the relatively conserved L1 genome. The most commonly employed PCR primer sets are the GP5+/6+ and MY09/11 systems. These can also be used in tandem in a nested assay format to increase sensitivity.¹⁶ The PCR product is then subjected to genotyping by a variety of methods, including restriction fragment length polymorphism (RFLP) and nucleotide sequencing. Infection with mixed types however would yield results that are difficult to interpret. A promising technique is the reversed line blot assay, using biotinylated primers for PCR, followed by hybridization with DNA of known HPV types immobilized in a line blot format. This assay has the advantages of being relatively simple to perform and able to detect multiple HPV types. The testing strategies described above are varied in sensitivity, specificity, detection of mixed genotypes, and ability to identify novel genotypes. Interpretation of study findings thus requires caution, since results from different methods are not strictly comparable. Serology does not have a role in HPV diagnosis. Its use is relatively recent and it is based on recombinant self-assembled virus-like particles as antigens. Serology assays are mainly performed in selected centres for population epidemiological studies.^{10,17}

Treatment

Since benign HPV lesions may regress spontaneously, treatment may not be indicated in all cases. Recurrence is common after treatment. Various modalities have been reported and no single therapy appears superior. Considerations for the choice of method include the location and extensiveness of lesions, side effects of treatment, availability of equipment and technical expertise, and cost.² Three main strategies are employed. Tissue ablation is the most commonly used strategy which could be applied to most body surfaces. Methods include cryotherapy with liquid nitrogen, surgical excision and laser ablation. Another strategy is chemical treatment, which can be applied topically or by intralesional injection. Agents reported include podophyllin, trichloroacetic acid, 5-fluorouracil and cidofovir. The third strategy, immunomodulation, has been employed with a view to enhancing cell-mediated immunity. Topical imiquimod is one of the more commonly used agents. Therapeutic vaccines as immunomodulators have been undergoing clinical trials.^{18,19} Further study is however required before their clinical role could be established. Review articles have been published on treatment of wart lesions and these provide comprehensive and up-to-date discussion on various modalities.^{2,20}

Prevention

Prevention is currently targeted mainly at avoiding direct contact with lesions, including safe sexual practices, and refraining from contact with potentially contaminated surfaces. A vaccine for HPV is not yet available, although various groups have evaluated candidate vaccines in both animal and clinical studies.² Since HPV type 16 has been found to be associated with the majority of cases of cervical carcinoma, most effort has been directed at preventing infection with this type. A recent placebo-controlled trial employing an HPV 16 virus-like-particle vaccine has shown promising results with 100% protective efficacy against

persistent HPV infection and cervical intraepithelial neoplasia on follow-up at two years.²¹ The vaccine targets the L1 viral capsid protein and prevention of infection is thought to be mediated through protective neutralising antibodies. It was well tolerated and generated high levels of antibodies against HPV 16 after a three-dose course, although HPV infection with other types could not be prevented. It is anticipated that this strategy, especially with the use of multivalent vaccines including other common high risk HPV types, may eventually reduce the incidence of cervical cancer.

Conclusion

HPV-related diseases are significant causes of morbidity and mortality in human. Understanding the pathogenetic mechanisms, especially in relation to the development of malignancies, has enabled a rational approach in screening, detection, management and prevention of this infection. The prospect of eventual reduction of the disease burden via various interventional strategies is promising.

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