

HUMAN PAPILLOMAVIRUS INFECTION AND CERVICAL CANCER: PATHOGENESIS AND EPIDEMIOLOGY

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Human papillomavirus (HPV) is one of the most common causes of sexually transmitted disease in both men and women worldwide. HPV is associated with a variety of clinical conditions that range from innocuous lesions to cancer. Genital HPV types are divided into high and low-risk types, according to the oncogenic potential. Molecular and epidemiologic studies have solidified the association between high-risk HPV types (especially HPV-16 and HPV-18) and cervical squamous cell carcinoma. HPV infection is often transient and self-limiting but uncommonly, infection persists and progress to high grade lesions and cancer. In addition to persistent high-risk HPV infection, other viral factors such as high viral loads, HPV variants, infections with multiple high-risk HPV types and genetic predisposition contribute to the development of cervical cancer. Although HPV is the primary etiologic agent of cervical cancer, it is not sufficient, and a variety of factors contribute to the cancer development. Risk factors include various aspects of sexual behavior (e.g. number of sexual partners, age of first sexual activity), smoking, long-term oral contraceptive use, immunosuppression, and presence of other sexually transmitted agents.

Keywords: Human papillomavirus; Cervical cancer; Cervical squamous cell carcinoma; Cervical intraepithelial neoplasia; Pathogenesis; Epidemiology.

INTRODUCTION

Papillomavirus are ubiquitous and have been detected in a wide variety of animals as well as in humans. More than 200 types of human papillomavirus (HPV) have been recognized on the basis of DNA sequence. Specific types of HPV tend to show some tissue tropism, and depending on the type of epithelium infected, HPV types are often referred to as “cutaneous” or “mucosal” types. In general, cutaneous types infect the keratinizing epithelium (especially the skin of the hands and feet), while mucosal types infect nonkeratinizing epithelium, primarily the anogenital tract epithelium, though they can also be found in the oral mucosa, conjunctiva and respiratory tract [1].

HPV is associated with a variety of clinical conditions that range from innocuous lesions to cancer (Table 1) [1]. Most HPV infections are benign. Infection of cutaneous epithelium can cause warts (plantar warts, common warts and flat warts). Skin warts are transmitted by direct contact with an infected tissue or indirectly by contact with virus-contaminated objects. In general, they resolve spontaneously within 1 to 5 years. Epidermodysplasia verruciformis, a rare genetic disease with HPV-associated warts on the trunk and upper extremities, can develop into invasive squamous cell carcinoma. Recurrent respiratory papillomatosis is primarily a disease of the larynx in young children but can also occur in adults. The infection in young children is thought to be acquired by passage through an infected birth canal. Respiratory tract lesions may undergo malignant transformation. Focal epithelial hyperplasia of the oral cavity (Heck’s disease) tends to regress spontaneously. Conjunctival papillomas associated with HPV have been described. HPV-6 and HPV-11 are the etiologic agents of external anogenital warts (condylomas), which occur in sexually active individuals. Although they are benign, genital warts are a significant problem in sexually active populations. Anogenital cancers are the most important diseases associated with HPV infections.

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HPV is one of the most common causes of sexually transmitted disease in both men and women worldwide. The incidence of new infections in the United States ranges from 1 million to 5,5 million per year, and the prevalence is estimated to be as high as 20 million [2]. Of the many types of HPV, about 30 infect the genital tract through sexual contact. Genital HPV types infect primarily the cervix, vagina, vulva, penis and anus. These genital-type HPVs are further divided into high and low-risk types, according to the association with genital tract cancer. Low-risk HPV types include types 6, 11, 42, 43, and 44, and usually cause benign anogenital warts. High-risk HPV types include types 16, 18, 31, 33, 34, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 70, and cause anogenital cancer [3]. Among the cancers attributable to high-risk HPV infection, cervical cancer has received the most attention. HPV-16, -18, -31, -45 account for more than 90% of cervical carcinomas [3]. Of these types, HPV-16 is the most often found, accounting for about half of the cervical cancer cases in the United States and Europe [3]. In addition, high-risk HPV types have been related with other genital cancers, such as carcinoma of vagina, vulva, penis and anus, and their precancerous lesions [4].

Table 1. Human papillomavirus types and clinical manifestations^a.

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

HPV: human papillomavirus.

^aData from reference 1.

^bOrder indicates relative frequency; bold type indicates most frequent association.

Cervical cancer and premalignant lesions constitute a major problem in women's health. Cervical cancer is the second most common cancer in women worldwide and is the most frequent cancer in many developing countries. Every year, 470.000 new cases of cervical cancer are diagnosed worldwide, and

about half of the afflicted women will die [5]. Although cervical screening has dramatically reduced the incidence of this disease in the developed world, it is still estimated that there will be 5.000 deaths from cervical cancer in the U.S. per year [5]. In areas of the world where most women do not have access to regular gynecological care and screening, cervical cancer is second only to breast cancer as a cancer-related cause of death [6].

The link between genital HPV infections and cervical cancer was first demonstrated in the early 1980s by Harold zur Hausen, a German virologist. Since then, the link between high-risk HPV types and cervical squamous cell carcinoma has become well known. In 1996, the World Health Association recognized HPV as an important cause of cervical cancer. HPV has been implicated in 99.7% of cervical squamous cell carcinoma cases worldwide [7]. The magnitude of the association between HPV and cervical carcinoma is higher than that for the association between smoking and lung cancer [8]. Adenocarcinomas of the cervix are also related to HPV (especially HPV-18), but the correlation is less pronounced and is age dependent. In women younger than 40 years, HPV was present in 89% of adenocarcinomas, whereas in women aged 60 years and older, HPV was observed in only 43% [9].

VIROLOGY

Papillomaviruses are members of the *Papovaviridae* family. HPV is a small, nonenveloped virus, with a diameter of 55nm. It has an icosahedral capsid composed of 72 capsomers, which contain at least two capsid proteins, L1 (major) and L2 (minor). Each capsomer is a pentamer of the major capsid protein. Each virion capsid contains several copies (about 12 per virion) of the minor capsid protein. The HPV genome consists of a single molecule of double-stranded, circular DNA containing approximately 7.900 bp associated with histones [10]. The genome is functionally divided into three regions. The first is a noncoding upstream regulatory region (URR). This region contains the p97 core promoter along with enhancer and silencer sequences that regulate DNA replication by controlling the transcription of the “early” and “late” regions. The URR region also contains the highest degree of variation in the viral genome. The second is the “early” region, which include the genes E1, E2, E3, E4, E5, E6, E7 and E8. This region is involved in viral replication and oncogenesis. Expression of the early gene products determines whether an HPV infection is active or latent, or leads to malignant transformation. The third is the “late” region, which encodes the L1 and L2 structural proteins for the viral capsid. HPV gene functions are showed in table 2.

Table 2. Human papillomavirus gene functions^a

Gene Category	Gene	Function
Early genes	E1	Viral replication
	E2	Modulation of transcription and replication
	E3	Unknown
	E4	Productive viral infections
	E5	Transforming properties
	E6	Oncoprotein; interaction with p53 protein
	E7	Oncoprotein; interaction with pRb protein
	E8	Unknown
Late genes	L1	Major capsid protein
	L2	Minor capsid protein

^aData from reference 4.

A new HPV type is defined as showing less than 90% homology to any of the known types on the basis of the E6, E7, L1 regions. If between 2% and 10% DNA divergence is present, the two viruses are considered subtypes of the same HPV type. When they show less than 2% divergence, the viruses are considered variants [11]. Some variants have different biological and biochemical properties important in cervical cancer. Eighty-six complete genomes of HPV have been characterized and about 120 are partially characterized [12].

PATHOGENESIS

Transmission of HPV occurs primarily by skin-to-skin contact. Basal cells of stratified squamous epithelium may be infected by HPV. Other cells types appear to be relatively resistant. It is assumed that the HPV replication cycle begins with entry of the virus into the cells of the basal layer of the epithelium. It is likely that HPV infection of the basal layer requires mild abrasion or microtrauma of the epidermis. 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 [13]. 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 [14].

1. Molecular biology

Cervical cancer is one of the best understood examples of how viral infection can lead to malignancy. The molecular mechanisms of oncogenic HPV infection are presented in figure 1. High-risk HPV types can be distinguished from low-risk HPV types by the structure and function of the E6 and E7 products. In benign lesions caused by HPV, viral DNA is located extrachromosomally in the nucleus. In high-grade intraepithelial neoplasias and invasive cancers, HPV-DNA generally is integrated into the host genome. Integration of HPV-DNA disrupts or deletes the E2 region, which results in loss of its expression [15]. This interferes with the function of E2, which normally down-regulates the transcription of the E6 and E7 genes, and leads to an increased expression of E6 and E7 genes. The function of the E6 and E7 products during a productive HPV infection is to subvert the cell growth-regulatory pathways and modify the cellular environment in order to facilitate viral replication [16]. The E6 and E7 gene products deregulate the host cell growth cycle by binding and inactivating two tumor suppressor proteins: the tumor suppressor protein (p53) and the retinoblastoma gene product (pRb). The HPV E6 gene product binds to p53 and targets it for rapid degradation [17]. As a consequence, the normal activities of p53 which govern G₁ arrest, apoptosis, and DNA repair are abrogated. Low-risk HPV E6 proteins do not bind p53 at detectable levels and have no effect on p53 stability *in vitro*. The HPV E7 gene product binds to pRb and this binding disrupts the complex between pRb and the cellular transcription factor E2F-1, resulting in the liberation of E2F-1, which allows the transcription of genes whose products are required for the cell to enter the S phase of the cell cycle [14]. The E7 gene product can also associate with other mitotically interactive cellular proteins such as cyclin E. [16]. The outcome is stimulation of cellular DNA synthesis and cell proliferation. The E7 protein from low-risk HPV types binds pRb with decreased affinity. Next, the E5 gene product induces an increase in mitogen-activated protein kinase activity, thereby enhancing cellular responses to growth and differentiation factors. This results in continuous proliferation and delayed differentiation of the host cell [16].

The inactivation of p53 and pRb proteins can give rise to an increased proliferation rate and genomic instability. As a consequence, the host cell accumulates more and more damage DNA that cannot be repaired, leading to transformed cancerous cells [18]. In addition to the effects of activated oncogenes and chromosome instability, potential mechanisms contributing to transformation include methylation of viral and cellular DNA, telomerase activation, and hormonal and immunogenetic factors [18].

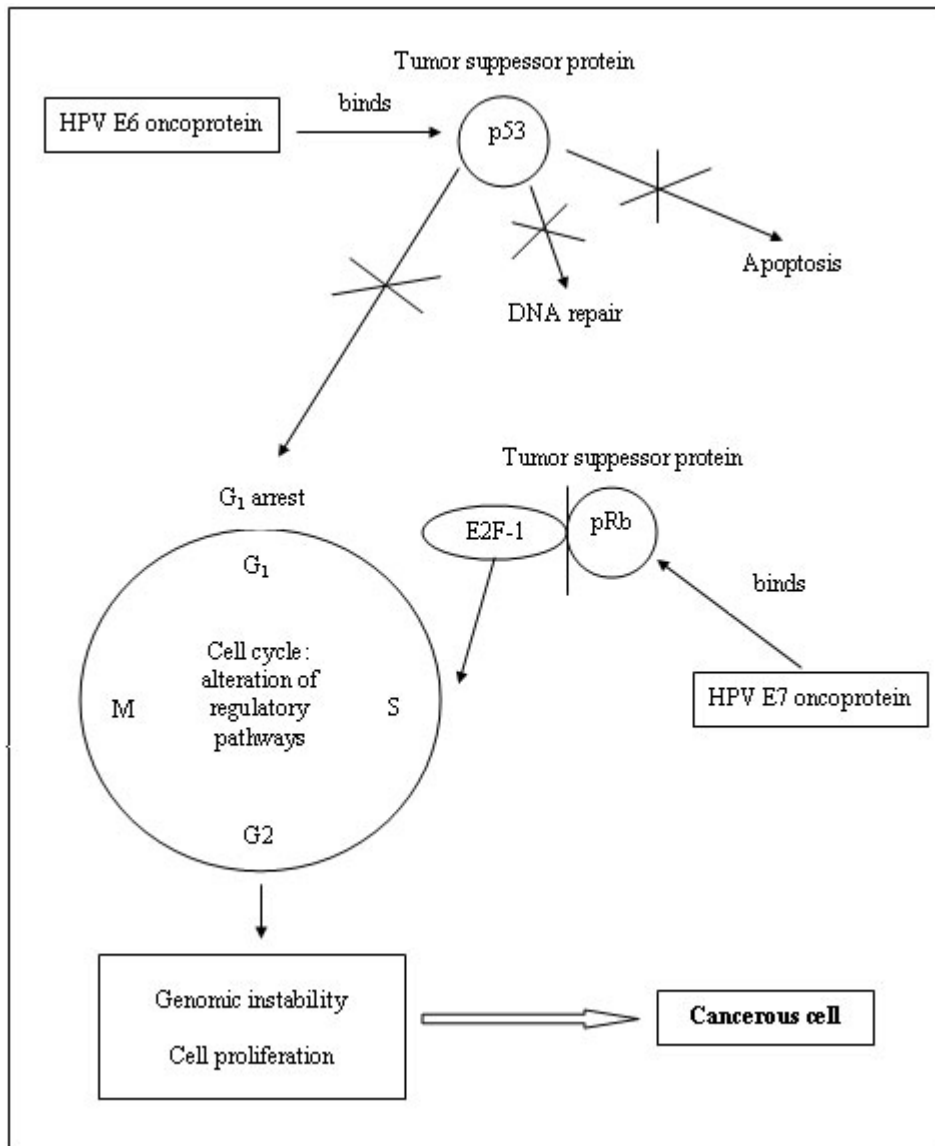


Figure 1. Molecular mechanisms of oncogenic HPV infection.

2. Natural history of cervical cancer

The pathogenesis of cervical cancer is initiated by HPV infection of the cervical epithelium during sexual intercourse. Even though a high percentage of sexually active young women are exposed to HPV infections, only a very small percentage go on to develop cervical cancer [19]. Several studies have suggested that most women successfully clear the HPV infection, presumably through the action of a competent immune system. Approximately, 90% of lesions regress spontaneously within 12 to 36 months [20]. Other factors such as genetic predisposition, frequency of reinfection, intratypic genetic variation within HPV type, coinfection with more than one HPV type and hormone levels may also influence the ability to clear an HPV infection.

The evidence for the importance of the host immune system in preventing the development of cervical disease comes from the analysis of HPV infections in human immunodeficiency virus (HIV)-positive women. HPV infections with high-risk viral types, persistence of HPV infection and the presence of squamous intraepithelial lesions are more common within this immunocompromised group than in immunocompetent women [21]. The host cellular immune response is mediated by cytotoxic T cells and requires the interaction of viral epitopes with histocompatibility class I molecules [22]. A humoral immune response also develops, but local levels of HPV-specific immunoglobulin G (IgG) and IgA in tissue do not correlate with clearance of virus [22]. However, systemic levels of HPV-specific IgA have been correlated with virus clearance. In contrast, systemic levels of HPV-specific IgG have been detected more frequently in patients with persistent HPV infection [22].

The natural history of cervical cancer is a continuous disease process that progress gradually from mild cervical intraepithelial neoplasia (CIN) to more severe degrees of neoplasia (CIN 2 or CIN 3) and finally to invasive cancer [23]. It is plausible that high-risk HPV infection occurs early in life, may persist, and in association with other factors promoting cell transformation, may lead to a gradual progression to more severe disease [24]. A model for the development of cervical cancer is presented in figure 2. Mild and moderate dysplasias are associated with continued viral replication and virus shedding, and most of these lesions spontaneously regress. Progression to high-grade lesions (CIN 2/3) and ultimately invasive cancer is usually associated with conversion of the viral genome from an episomal form to an integrated form, along with inactivation or deletion of the E2 region and expression of the E6/E7 product genes. Some investigators have correlated HPV type with different degrees of CIN and have suggested that CIN 1 and CIN 2/3 are distinct processes, with CIN 1 indicating a self-limited sexually transmitted HPV infection and CIN 2 or CIN 3 being the only true cervical cancer precursor [23]. Progression to cancer generally takes place over a period of 10 to 20 years. Some lesions become cancerous more rapidly, sometimes within two years [23].

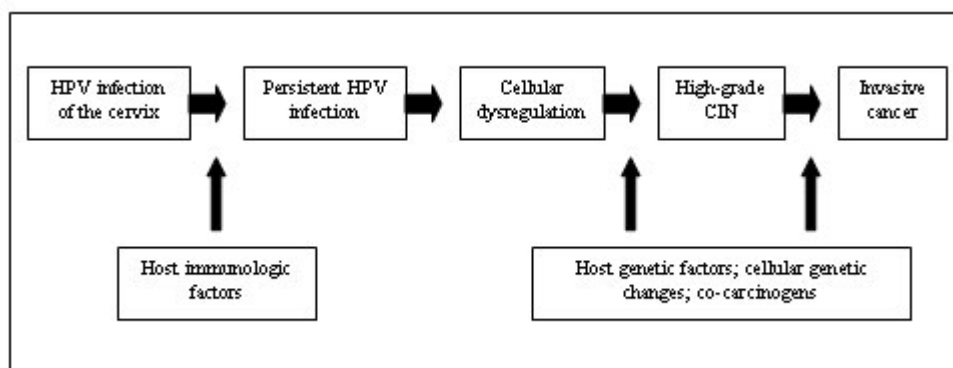


Figure 2. Model for the development of cervical cancer.

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 act in concert with cancer-associated HPV types.

1. Sexual factors

Numerous studies clearly indicate that the risk of contracting genital HPV infection and cervical cancer is influenced by sexual activity. An individual is at greater risk of becoming infected with HPV if he or she had multiple sexual partners at any time or is the partner of someone who has had multiple sexual

partners [25]. Sexual activity at an early age also have an increased risk of HPV infection, as does a history of other sexually transmitted diseases, genital warts, abnormal Pap smears or penile cancer in an individual or sexual partner [5]. Condom usage may not adequately protect individuals from exposure to HPV since the virus can be transmitted by contact with infected tissues that are not protected by a condom.

In addition to sexual activity, age is an important determinant of risk of HPV infection. Most cervical cancers arise at the squamocolumnar junction between the columnar epithelium of the endocervix and the squamous epithelium of the ectocervix. At this site, there are continuous metaplastic changes. The greatest risk of HPV infection coincides with greatest metaplastic activity. Greatest metaplastic activity occurs at puberty and first pregnancy and declines after menopause. The HPV prevalence reaches its peak in young adults (18 to 30 years of age) and declines at older ages [26]. As many as 46% of college women may have an HPV infection of the genital tract [27]. However, cervical cancer is more common in women older than 35 years, suggesting infection at a younger age and slow progression to cancer.

2. Viral factors

Persistent cervical infection (often defined as an infection that is detected more than once in an interval of 6 months or longer) with an oncogenic HPV type (especially HPV-16 and HPV-18) is the most important risk factor for progression to high-grade dysplasia and invasive cancer [24]. The risk of progression depends on the HPV type. A 4-6 year follow-up of 1.643 women with normal cytology showed that women with a positive PCR high-risk HPV DNA test at baseline were 116 times more likely to develop CIN 3 than women with a negative DNA test [28]. The risk of progression for HPV-16 and HPV-18 is greater than for other HPV types, approximately 40% [29].

It has been proposed that the viral load correlates directly with the severity of disease. Studies using quantitative type-specific PCR for high-risk HPV and low-risk HPV have shown that HPV-16 can reach much higher viral loads than the other types, and that only for HPV-16 high viral loads correlate with increased severity of cervical disease [30]. Other studies using Hybrid Capture IITM (Digene Diagnostics, Gaithersburg, MD) have shown an increased viral load of high-risk HPV types in high-grade lesions [31]. However, high-risk HPVs are able to induce malignant tumors even when they are present at low levels.

An important emerging factor in the development of cervical neoplasia is the role of HPV variants. HPV variants differ in biological and chemical properties and pathogenicity [32]. The oncogenicity of specific HPV variants appears to vary geographically and also with the ethnic origin of the population studied. Based on sequence variation of L1, L2 and URR regions of HPV-16, five variants have been defined for HPV-16: European (E), Asian (As), Asian-American (AA), African-1 (Af1) and African-2 (Af2). Asian-American variants might have enhanced oncogenic activity compared to European isolates due to an increased transcriptional activity [33].

Several studies have shown that infections with multiple types of HPV can occur [33, 34]. The majority of multiple infections contain two HPV types, but samples with two, three, four or five types were also seen [33]. The presence of multiple HPV types tended to increase with the severity of cervical disease. Multiple HPV types, usually with at least one type classified as high-risk, were found in 12% of patients with normal cytology and in 35% of patients with mild or moderate dysplasia [34].

3. Nonviral factors

The primary immune response to HPV infection is cell mediated; therefore, conditions that impair cell-mediated immunity such as renal transplantation or HIV disease, increase the risk of acquisition and progression of HPV [35, 36]. The URR region of HPV contains sequences similar to the glucocorticoid responsive elements that are inducible by steroid hormones such as progesterone (the active component of oral contraceptives). Long-term use of oral contraceptives is a significant-risk factor for high-grade cervical disease according to some studies [37, 38]. Cervical cancer risk also seems to be independently

influenced by other variables including current smoking and parity [38]. Local immune suppression induced by smoking and the mutagenic activity of cigarette components have been demonstrated in cervical cells, and may contribute to persistence of HPV or to malignant transformation similar to that seen in the lung [39]. It appears that smoking is the most important risk factor independent of HPV infection for higher grades of cervical disease [38]. Multiple pregnancies were a significant independent risk factor among women with histopathologic evidence of HPV infection in biopsy specimens and among women with CIN 2/3 [38]. Other factors such alcohol consumption and diet have not been well established.

There has been some suggestion that sexually transmitted viruses may serve as cofactors in the development of cervical cancer. It has been postulated that coinfection with herpes simplex virus type 2 may play a role in the initiation of cervical cancer [40]. Cytomegalovirus (CMV), human herpesvirus 6 (HHV-6) and HHV-7 have also been detected in the cervix. Coinfection offers the opportunity for these viruses to interact with HPV. Recent studies using PCR to detect CMV, HHV-6 and HHV-7 in women with abnormal cytologic test results indicate that these viruses are only bystanders rather than cofactors in the development of cervical cancer [41]. *Chlamydia trachomatis* infection has been associated with cervical cancer, but the meaning of this association remains unclear. Some authors have suggested that the association between *C. trachomatis* infection and cervical cancer may be due to an effect of *Chlamydia* infection on persistence of high-risk HPV [42].

Genetic predisposition was found to be a great component in cervical cancer [43]. Genetic heritability was found to account for 27% of the effect of underlying factors for tumor development. Heritability could affect many factors contributing to the development of cervical cancer, including susceptibility to HPV infection, ability to clear HPV infection, and time to development of disease. The effect of shared familial environment was shown to be small at 2% and was found only between sisters and not between mother and daughter [43].

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