Role of the human papilloma virus in the development of cervical intraepithelial neoplasia and malignancy

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Human papilloma virus (HPV) is a public health problem as a sexually transmitted disease and as a critical factor in the pathogenesis of various cancers. The clinical manifestations, epidemiology, and virology that are critical to understanding the process of cervical dysplasia and neoplasia are reviewed. A discussion of the cervical transformation zone and the classification of cervical dysplasia and neoplasia leads into the importance of the Papanicolaou smear in prevention of potentially devastating sequelae of this virus. The role of the immune system in the progression of the disease and how it relates to vaccines, as well as treatment and prevention of HPV, are reviewed.

Viruses, from the Latin “slime” or “poisonous juice” were not understood as unique entities until the 19th century. The major development that lead to understanding viruses occurred in 1884 when Chamberland created a filter with pores small enough to retain bacteria, yet large enough for viruses to pass through. A century later, in the 1950s, a variety of viral particles were documented by electron microscopy.

Papilloma virus was initially isolated from cottontail rabbits in 1933. In 1935 it was discovered that papillomas induced by papilloma virus had the potential to transform into malignant processes. Squamous cells that developed from human papilloma virus (HPV) lesions were noted in 1956. The authors called these cells koilocytic atypia or koilocytosis, meaning “hollow”, from the Greek word koilos. Before the invention of cloning techniques in the 1970s, investigation of HPV was difficult since the virus does not grow in culture. Zur Hausen cloned the potentially malignant HPV 16 in the 1980s. By the end of the 20th century, over 100 types of HPV were identified.

HPV is recognised as a public health problem for its role as a sexually transmitted disease and also as a critical factor in the pathogenesis of various cancers. HPV is a crucial element in the development of cervical cancer, the third most common malignancy in women. The cause and effect relationship between HPV and cervical cancer is compelling. HPV DNA has been found in 90% of cervical cancer and 50% of vulvar cancer. Fifty per cent of young, sexually active women are infected with HPV types that may promote the development of cancer. In fact, HPV 16 and 18 have been classified as “carcinogenic” by the World Health Organisation International Agency for Research on Cancer.

CLINICAL PRESENTATION
HPV infection is initially asymptomatic and transmission between people occurs before overt expression of the virus is seen or felt. Clinically, HPV infects the basal cells of the epithelium of skin and mucous membranes. Because HPV may affect sites where there are epithelial cells, infections have been documented in the oral mucosa, oesophagus, larynx, trachea, conjunctiva, as well as genital and anal areas. HPV has not been identified in gastrointestinal mucosa.

Infection may present in various ways. Latent HPV may be asymptomatic identified only by molecular biology techniques, or subclinical, seen on colposcopy. The virus may present as hyperplastic, hyperkeratotic warts or dysplastic lesions that may undergo neoplastic transformation.

Non-genital warts may be passed on by fomites or direct contact with even a small area of broken skin. Alternatively, genital warts are passed on through direct contact with the lesion. Genital warts in females appear initially on the posterior introitus and adjacent labia; the warts then spread to the vulva, and eventually to the vagina and cervix. Genital warts can cause intense discomfort with associated pruritis, bleeding, and secondary infection caused by superficial injury due to scratching.

Perhaps more important than the initial wart lesions that recur over the years, are the consequences of the latent infection with this virus—that is, dysplasia, neoplasia, and cervical cancer that occurs in a significant portion of these women. Important questions to ask include: Why do some women carry HPV for years with minimal sequelae while others are afflicted with carcinoma in situ? How can we prevent the spread of this potentially fatal virus? What is the role of the Papanicolaou (PAP) smear in the prevention of the more detrimental outcomes of this virus?

Epidemiology
Just under half of the 100 HPV types identified infect the genital tract. Of these viral types only a small number have been detected in malignant lesions—that is, HPV 16, 18, 26, 27, 30, 31, 33–35, 52, 58, 59, and 70. The most commonly identified types in cervical lesions are HPV 16 and 18, with HPV 16 accounting for 50% of cervical cancers.

Abbreviations: ASCUS, atypical squamous cells of undetermined significance; CIN, cervical intraepithelial neoplasia; HPV, human papilloma virus; HSIL, high grade squamous intraepithelial lesion; LSIL, low grade squamous intraepithelial lesion; PAP, Papanicolaou (smear)
Box 1: Cervical cancer

Risk factors
- Increased number of sexual partners.
- Increased frequency of intercourse.
- Early age of first intercourse.
- Prostitution.
- Sexual behaviour of male partner.

Associated risk factors
- Tobacco smoking (nicotine metabolites identified in cervical mucus of female smokers).
- Use of oral contraceptives.
- Infection with other sexually transmitted diseases.
- High parity.
- Lack of certain nutritional factors (that is, as vitamin C or β-carotene).

Virology
The HPV virus is a non-enveloped, double stranded, circular DNA virus made of 7900 base pairs from the family Papovaviridae, genus papillomavirus. It has an icosahedral capsid made of 72 capsomeres and is 50–55 nm in diameter. Understanding the genomic organisation of HPV is crucial in understanding the oncogenic process it induces in the development of cervical dysplasia. Significant regions include the early region (E), the late region (L), and the long control region. E1 and E2 are responsible for viral DNA replication and gene expression. E1 encodes the capsid protein which makes up 95% of the viron mass; whereas E2 encodes for the minor capsid protein. E6 and E7 in essence immortalise the human keratinocytes. E6 mediates degeneration of p53 tumour suppressor protein while E7 binds retinoblastoma gene products. The sequence of events which disturb the normal apoptotic process of cervical cells, thus transforming them into immortal dysplastic cells, is as follows: circular viral DNA integrates E1/E2 region; as E2 is split and inactivated E6 and E7 products are enhanced; this leads to inactivation of p53 and pRB which in turn leads to cell cycle progression and immortalisation of normal cervical cells. The HPV virus has an incubation period of three to four months. The virus infects the basal cells, virens assembly in the nucleus, and subsequently are released as keratinocytes. There is proliferation of all epidermal layers, except the basal layer. Products of this proliferation include acanthosis, parakeratosis, and hyperkeratosis that then clinically manifest as condylomata acuminata or genital warts.

CERVICAL DYSPLASIA
The cervical transformation zone is of particular significance as it is the location where cervical dysplasia most often occurs. The cervix is made up of two histologically unique parts. The endocervix, which is predominantly comprised of columnar cells, and the exocervix, which is made up of squamous cells. The transformation zone, also called the squamocolumnar junction, is the area of the cervix where the endocervix and exocervix meet. Starting during puberty, the columnar cells of the endocervix slowly transform into squamous cells, thus it is said that the exocervix “moves in” towards the cervical os.

Classification of dysplastic lesions is also an important issue. Depending on the time of publication, various sources use different descriptions and classification systems. In the 1940s, cervical abnormalities were described by Papanicolaou who devised a five class system (I to V), which included mild, moderate, and severe dysplasia, carcinoma in situ, and cancer. In the late 1960s, Richart created a new classification of dysplasia where changes were termed cervical intraepithelial neoplasia (CIN) I, II, and III. It was thought that a progression of events occurred as patients advanced sequentially through these stages on a continuum from CIN I to carcinoma in situ.

As cervical dysplasia was studied more closely it became evident that it did not follow a straightforward progression. With a diagnosis of CIN I, about 1% progress to invasive carcinoma. The transformation from CIN I to CIN II occurs very quickly, with about 5% on CIN II progressing to invasive cancer. Evidence indicates that CIN III does in fact have a significant risk (>12%) of progressing to invasive carcinoma if left untreated. The treatment for CIN II and III is essentially the same and, therefore, clinically did not warrant separate categories. In addition, this system of classification was not utilised uniformly throughout academic institutions and other more descriptive classifications continued to be used. A uniform classification system was needed for algorithms of treatment protocols and for groupings in scientific studies.

Created in 1988 and revised in 1991, the Bethesda system for rating cytology accomplished these goals and continues to be in current use. This system includes the following classes: atypical squamous cells of undetermined significance 39, 40, 42–45, 51–59, 61, 62, 64, 66–69, and 71–74. HPV 6 and 11 are responsible for benign warts, two of these types include HPV 6 and 11.

Other risk factors for the development of cervical cancer are
- Lack of certain nutritional factors (that is, as vitamin C or β-carotene).
- High parity.
- Use of oral contraceptives.
- Tobacco smoking (nicotine metabolites identified in cervical mucus of female smokers).
- Infection with other sexually transmitted diseases.
- Early age of first intercourse.
- Increased frequency of intercourse.
- Increased number of sexual partners.
- Sexual behaviour of male partner.
- Prostitution.
- High parity.

Table 1: The Bethesda system for rating cytology

<table>
<thead>
<tr>
<th>Bethesda system</th>
<th>Description</th>
<th>Papanicolaou classification</th>
<th>Cervical intraepithelial neoplasia</th>
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</thead>
<tbody>
<tr>
<td>ASCUS</td>
<td>Atypical squamous cells of undetermined significance</td>
<td>Squamous atypia</td>
<td>Papanicolaou class II</td>
</tr>
<tr>
<td>LSIL</td>
<td>Low grade squamous intraepithelial lesions</td>
<td>Mild dysplasia, koliocytic or condylomatous atypia</td>
<td>Papanicolaou class III</td>
</tr>
<tr>
<td>HSIL</td>
<td>High grade squamous intraepithelial lesion</td>
<td>Moderate to severe dysplasia</td>
<td>Papanicolaou class IV</td>
</tr>
</tbody>
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CIN I
CIN II
CIN III
Carcinoma in situ
(ASCUS), low grade squamous intraepithelial lesions (LSIL), high grade squamous intraepithelial lesions (HSIL), and carcinoma in situ (see table 1).  

**ROLE OF THE PAP SMEAR**

The Papanicolaou smear is based on the work of a Greek physician, George N Papanicolaou.  Papanicolaou first began to study normal and abnormal vaginal cytology in the 1920s.  He published his findings with Herbert Traut, a gynaecological pathologist, in 1941 in *The diagnostic value of vaginal smears in carcinoma of the uterus.*  Named after its creator, today the vaginal smear is known as the PAP smear.

Unfortunately, there has never been an evaluation of PAP smear screening in a randomised control trial.  The data regarding screening is based on differences seen geographically over the time that the PAP smear has been used.  Over-all, the PAP has decreased the rate of stage II squamous cell carcinoma in women that are regularly screened but has not been shown to decrease the rate of adenocarcinoma or adenocarcinoma in situ.  The American College of Obstetrics and Gynecology recommends that PAP smear screening should start at age 18 or when the woman becomes sexually active.  The recommendation is to continue annual screening until three normal PAP smears have been obtained; at this time, if the physician prefers, the women may be tested less frequently.  There is no consensus as of yet to what age to stop PAP smear screening, but since cervical cancer may be diagnosed postmenopausally, individual risk factors must be taken into consideration.  The American Academy of Family Physicians recommends discontinuation of PAP smear screening after age 65 if the woman does not have a history of positive PAP smears.  In women who have had hysterecomies the incidence of cervical cancer is extremely low. Therefore if the uterus was removed for benign reasons, the cervical epithelium was completely removed, and the woman had no previous abnormal PAP smear findings, screening may be discontinued.

**ROLE OF THE IMMUNE SYSTEM IN THE PROGRESSION OF HPV TO CERVICAL DYSPLASIA**

Why does cervical dysplasia undergo a malignant transformation in some women but not others?  It appears that the immune system plays a significant part in the regression or progression of cervical dysplasia.  One study suggested that CD8 cells against HPV antigen were more prevalent in women diagnosed postmenopausally, and individual risk factors must be taken into consideration.  The American Academy of Family Physicians recommends discontinuation of PAP smear screening after age 65 if the woman does not have a history of positive PAP smears.  In women who have had hysterecomies the incidence of cervical cancer is extremely low. Therefore if the uterus was removed for benign reasons, the cervical epithelium was completely removed, and the woman had no previous abnormal PAP smear findings, screening may be discontinued.

**PREVENTION**

First, it is important to counsel patients on the environmental risk factors that may predispose them to becoming infected with the HPV virus.  If a patient decreases the number of sexual partners she has, she will then decrease the probability of becoming infected with HPV. In addition, although HPV may be spread even if a barrier method is used, the use of barrier contraceptives decreases the probability that the patient will become infected.  Annual PAP smears have become a crucial tool in decreasing mortality from cervical cancer.  Mortality from cervical cancer has decreased by over 40% since the start of PAP smear screening in the United States.  This decrease in mortality occurs because the lesions are detected early on when they are still treatable.

Promising future forms of treatment are various HPV vaccines.  There are several forms of HPV vaccines/cervical cancer vaccines that are currently under investigation.  The transfer of cytotoxic T-lymphocytes has been successful in eliminating certain tumours in mice.  Peptide based vaccines have been shown to activate antigen specific cytotoxic T-cells.  Virus-like particles, for example containing only the major capsid protein, may potentially be used as a prophylactic vaccine and have shown to produce humoral immunity in mice.  Vaccination with a vector encoding for HPV has produced humoral and cell mediated responses in mice which DNA vaccines have shown a similar response in rabbits.  Many of these studies are still at the level of animal based models therefore it may be years before scientists create what would be the ideal HPV vaccine; one which would have preventative as well as therapeutic faculties.

**CONCLUSION**

In conclusion, HPV is a significant public health problem as a sexually transmitted disease and more importantly as a crucial contributing factor to the development of cervical cancer.  Consequently, PAP smear screening has become a crucial part of women’s health as early detection of cervical dysplasia has significantly reduced the morbidity and mortality of cervical cancer in the United States.  Therefore, it is not only necessary for physicians to continue PAP smear screening on women who present for the “annual well women exam”, but it is also vital to target women who present to the clinic for other health problems; women who would otherwise not be screened for potentially devastating sequelae.

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REFERENCES