Human Papillomaviruses

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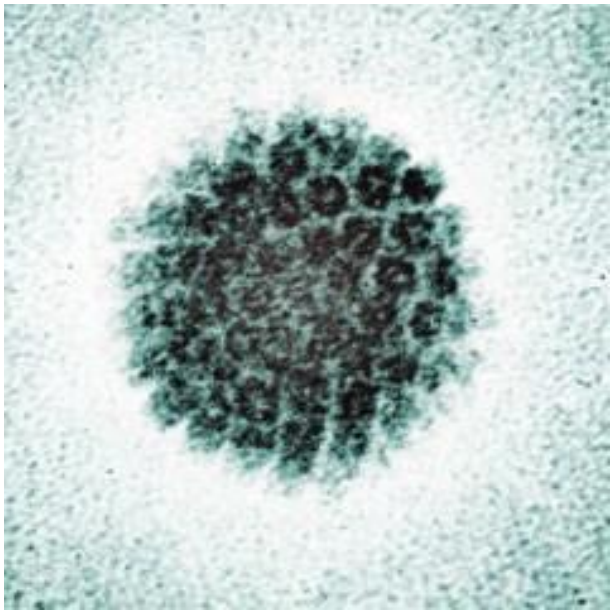
Contents of Teaching in Medical Virology Lecture:

Human papillomaviruses

This family of viruses exclusively infects epithelial surfaces where they cause hyper proliferative lesions. Some HPV types infect keratinized epithelium and others mucosal surfaces. The typical lesion caused by HPV is a wart. HPV is of major medical importance because some HPV types are causally linked to various cancers, especially cancer of the cervix.



Virology

Family: Papillomaviridae

Genus: Various, e.g. Alphapapillomavirus

Species: Human papillomavirus xx (82 genotypes assigned, but more than 100 distinct viruses)

Structure: Small, unenveloped, circular dsDNA

Papillomavirus classification system

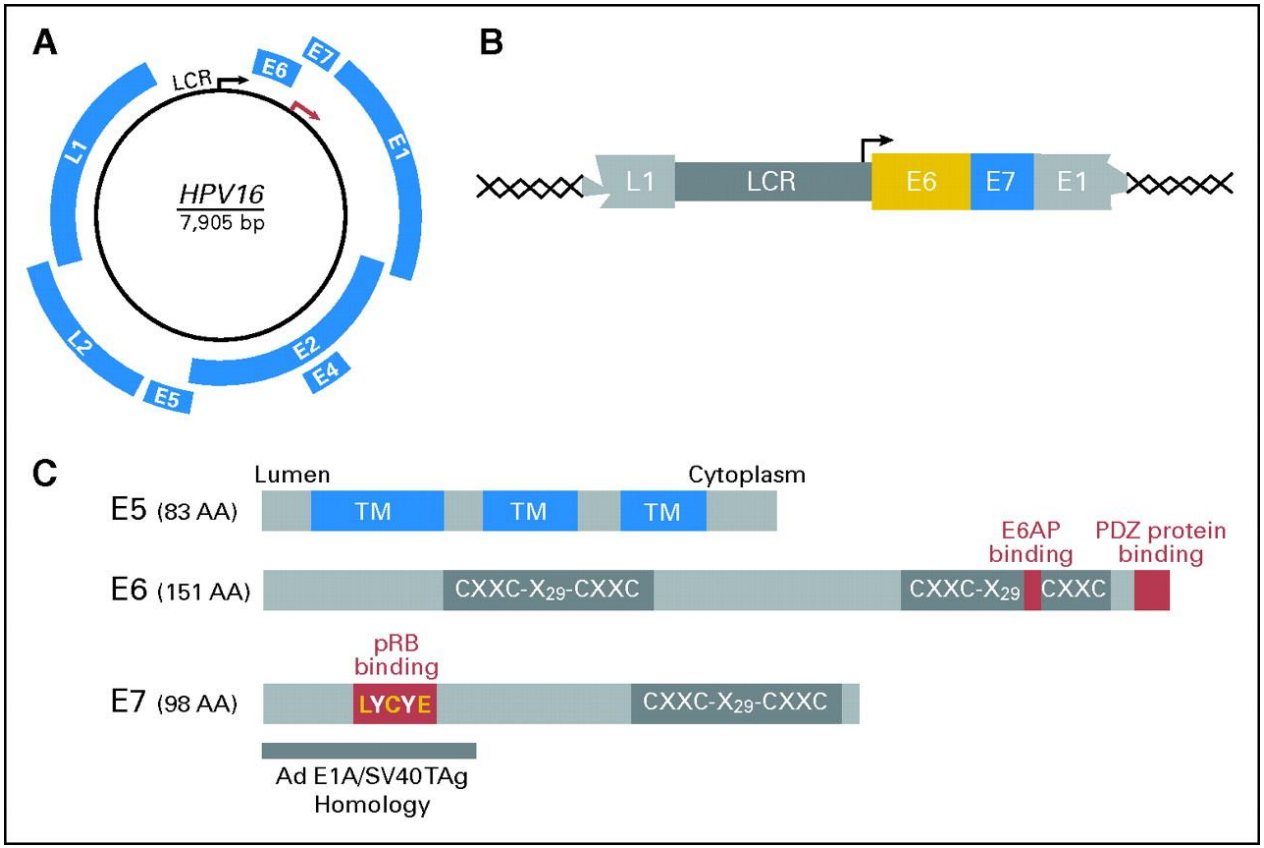
Overview:

Taxonomic level	Criteria	Number
Genera	Less than 60% nt identity in entire L1 ORF	18 genera (alpha to sigma) 2 additional new genera, yet unnamed (containing TtPV2 and RaPV1 respectively)
Species	Between 60% and 70% nt identity in L1	From 1 species (in most genera) up to 15 species (in genus alpha)
Genotypes	Between 71 and 89 % nt identity in L1	Currently 120 papillomavirus genotypes completely genomically characterized and available in Genbank (98 human: HPV; 32 non-human)
Subtypes	Between 90 and 98 % identity in L1	Very rare
Variants	More than 98 % identity in L1	

Virus replication

HPV replicates exclusively in stratified squamous epithelium. It requires differentiating epithelium to undergo a complete replication cycle. Infection is initiated when the virus gains access to the basal epithelial cells (through abrasion). In the basal layer, early non structural viral proteins are expressed (including E6 and E7) which stimulate the infected cells to proliferate faster. This is followed by replication of the viral genome. As the epithelial cells mature (move up the epithelial layer), there is a switch to expression of the viral structural genes (L1 and L2). Production of structural viral proteins is initiated and new virus particles assemble and are shed as the infected cells desquamate.

Epithelium is an immune-privileged site and the virus evades immune recognition and may persist for many months (even years) in the same individual.



Clinical disorders due to HPV infection

Most HPV infections are clinically silent. Only a minority of people develop clinically apparent lesions.

Warts

Cutaneous warts are caused by HPV types 1, 2, 3, 4, 5 and 8. They are benign painless proliferative lesions that occur on the skin at sites of abrasion such as hands, feet, knees and elbows. They are very common, especially in children over the age of five years. They typically persist for months, but eventually regress in immuno-competent people.

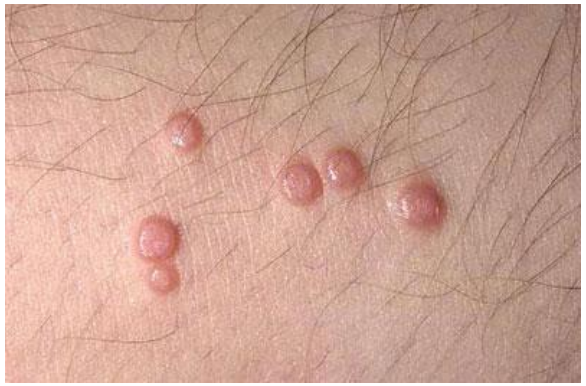
Mucosal warts: These are mainly due to HPV 6 and 11. Lesions are most commonly seen in genital mucosa (usually sexually transmitted), but may occur in oropharynx and they are also the cause of laryngeal papillomas. Like cutaneous warts, they may persist for months or years and although they are benign they can cause much distress due to disfigurement of the genital area.

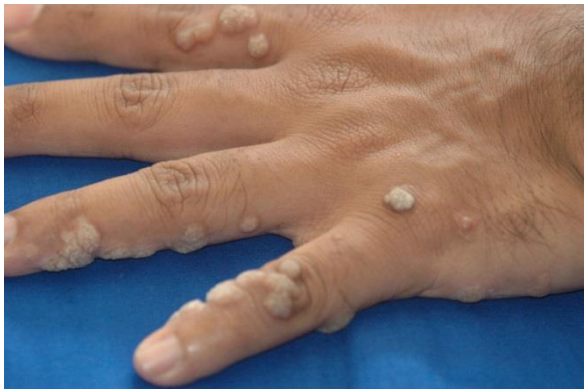
Epidermodysplasia verruciformis

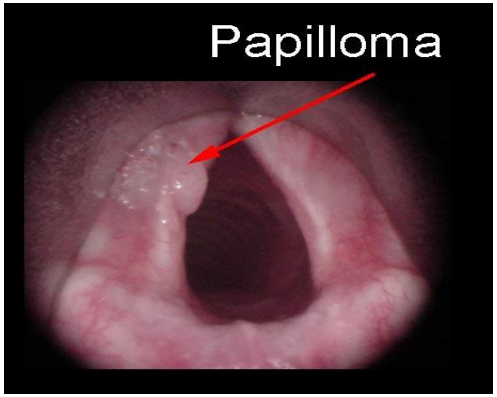
This occurs with a rare immunodeficiency state where the patients are highly susceptible to HPV infection. They present with chronic extensive skin infection with multiple HPV types. Some skin lesions may undergo malignant transformation.

Laryngeal papillomatosis

This condition, most commonly seen in infants and young children is due to infection with HPV 6 or 11. Warts develop in the larynx, especially on the vocal cords. Infection is usually acquired during passage through infected birth canal. Affected children develop hoarseness and stridor and airway obstruction. Treatment involves repeated surgical removal of the growths.

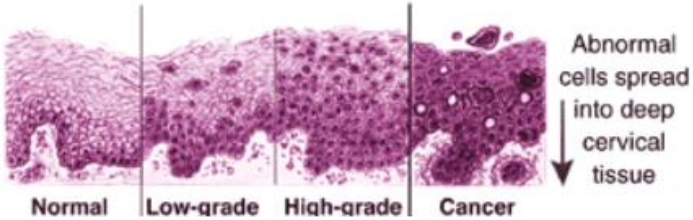






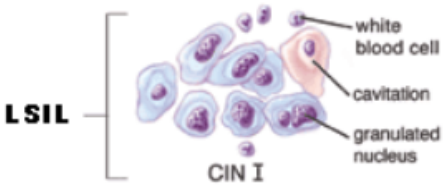
ASCUS ~ Atypical squamous cells of undetermined significance

This diagnosis means that some of the cells on your Pap smear did not look entirely normal but did not meet diagnostic criteria for a lesion. Your doctor may either repeat your Pap smear, or perform a colposcopy (link to above). The lab may test your Pap smear specimen for HPV.



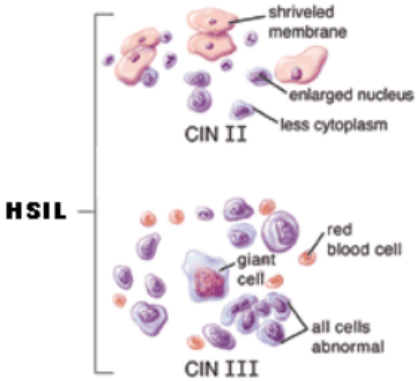
LSIL ~ Low-grade squamous intraepithelial lesion

This diagnosis means there are early changes in the size and shape of the cells. LSILs are often associated with HPV, which may also cause genital warts. These lesions, in women with intact immune systems, often resolve without intervention within 18 to 24 months. Low-grade lesions may also be called mild dysplasia, or CIN1. If it is your first abnormal Pap smear, your doctor will likely recommend a colposcopy (link to above).

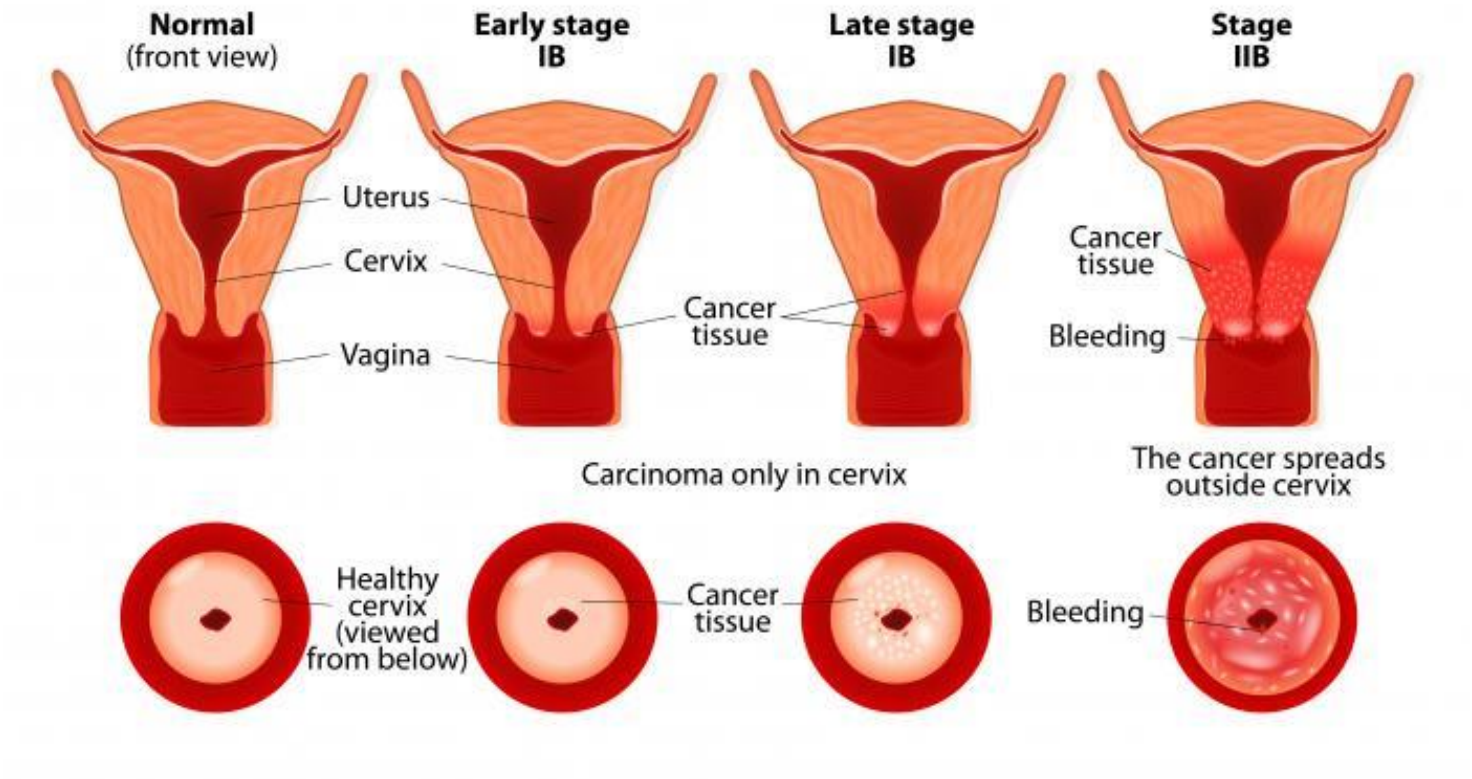


HSIL ~ High-grade squamous intraepithelial lesion

This diagnosis means the cells appear very different from normal cells. These precancerous lesions are more severe than with LSIL, but involve cells on the surface of the cervix. They may also be called moderate or severe dysplasia, or CIN 2 or 3. The treatment for HSIL is to remove the abnormal tissue. This can be done in several ways. See the treatment section for more information.



CERVICAL CANCER



Epidemiology of genital HPV infections

Mucosal-tropic HPVs are mainly sexually transmitted. The incidence of infection (in both males and females) is highest shortly after onset of sexual activity and declines to a low level at about 30 years of age after which it rises again. Prevention is difficult as the vast majority of infections are asymptomatic. Condoms do not work very well as prevention as the virus may infect any part of the genital mucosa. Around 80% of women are infected with HPV in their life time. Re-infections with the same or another HPV type can occur throughout life. Most of these infections are cleared with the development of a type specific immune response, but a small proportion of patients may develop persistent infection. Persistent infection with multiple HPV types is common in HIV infection due to compromised host immunity. Persons who fail to clear infection are at risk for malignancies in the genital tract.

Table 4
Detection of HPV-DNA in esophageal squamous cell carcinomas.

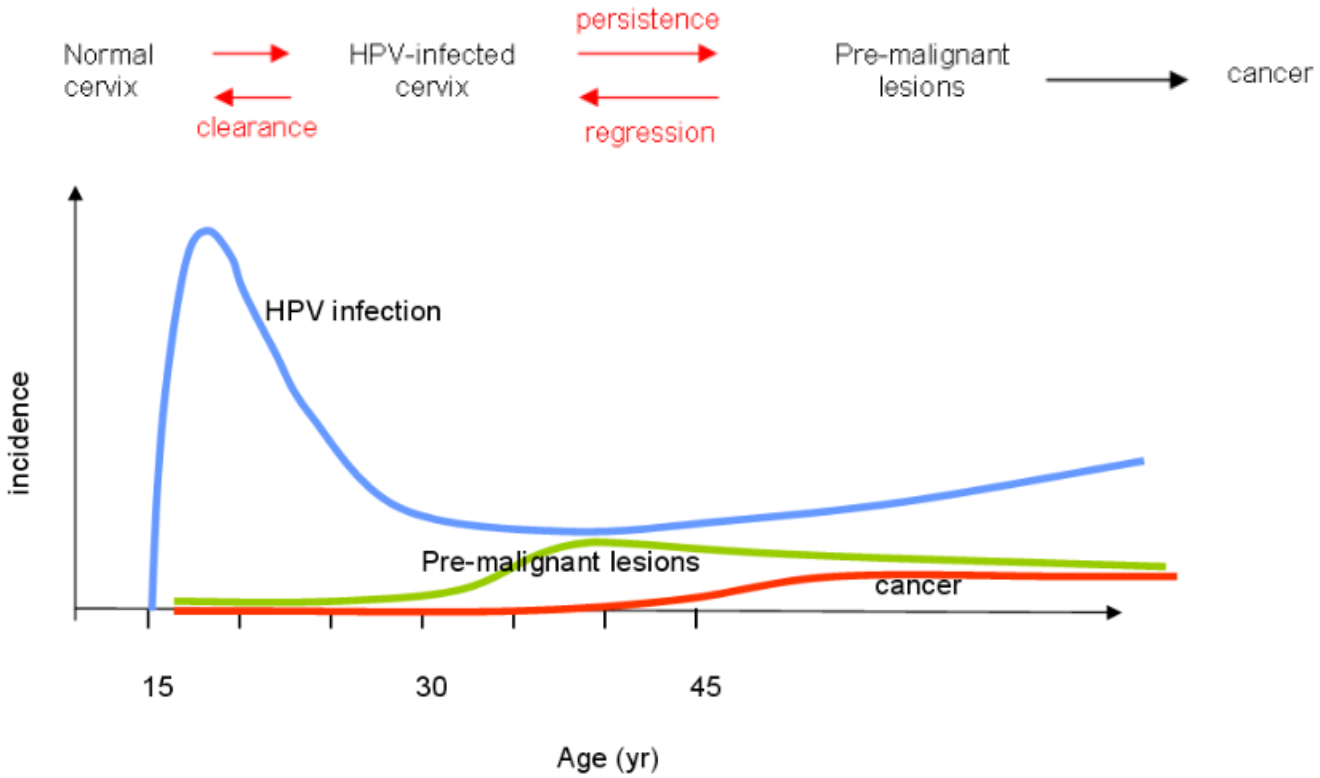
Author, reference	Year	Country (region)	Cases, *n*	HPV positive cases, *n*	Rate of HPV isolation (%)
Those defending the opinion that "HPV infection is an important etiological factor in esophageal SCCs"					
Chang et al. [20]	2000	China	700	118	16.9
Matsha et al. [21]	2002	South Africa	50	23	46
Farhadi et al. [22]	2005	Iran (Tehran)	38	14	36.8
Yao et al. [23]	2006	China (Henan immigrants and native population)	82	32	39
Souto Damin et al. [24]	2006	Brazil	165	26	15.75
Lu et al. [9]	2007	China (Kazakh ethnic group)	60	20	30
Ding et al. [25]	2010	China	17	8	47
Cui et al. [14]	2011	China (Linzhou city)	18	18	100
Yahyapour et al. [26]	2012	Iran (North of Iran and around the Caspian Sea)	117	49	27.7
Guo et al. [27]	2012	China	300	93	31
Gupta et al. [13]	2012	India	44	17	38.6
Wang et al. [28]	2013	China (Northern China)	92	19	20.65
Vaiphei et al. [29]	2013	India	23	20	83
Liu et al. [30]	2014	China (Northern China, Henan State)	78	54	69
Those defending the opinion that "HPV infection does not play a dominant etiological role or is a minor etiological factor in SCCs of esophagus"					
Lambot et al. [31]	2000	Belgium	21	1	4.8
Antonsson et al. [7]	2010	Australia	222	8	3.6
Abdirad et al. [32]	2011	Iran	93	8	8.6
Herbster et al. [33]	2012	Brazil (Southeastern Brazil)	264	38	13
Tao Liu et al. [34]	2012	China (Uighur, Kazakh, Han ethnic group)	253	52	20
Schäfer et al. [35]	2013	South Africa	114	10	9
Those defending the opinion that "the role of HPV infection in esophageal SCC is debatable, absent, or results must be verified by further studies"					
Kamath et al. [36]	2000	America	22	0	0
White et al. [37]	2005	Southwestern Kenya	29	0	0
Mir et al. [38]	2007	India (Kashmir)	62	0	0
Koh et al. [39]	2008	Korea	139	0	0
Tornesello et al. [6]	2009	Italy	36	10	27.8
Patel et al. [15]	2011	Kenya	28	0	0
Noori et al. [40]	2012	Iran	92	0	0

HPV and carcinogenesis

Certain mucosal tropic HPV types can cause cancer in healthy people. These are referred to as high risk HPV types. High risk HPV types are responsible for various cancers of the genital tract including penile, vulval, anal, vaginal and cervical cancer.

Cancer of the cervix is the second commonest cancer in women world wide and the commonest cause of cancer deaths in women in developing countries. Around 15 high risk HPV types may cause this cancer, but the most commonly implicated are HPV 16 which accounts for 50% of cervical cancers and HPV 18 responsible for 10-20% of cervical cancer worldwide. (The remaining 30% are due to HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 63, 68, 82).

HPV infection and cancer development:

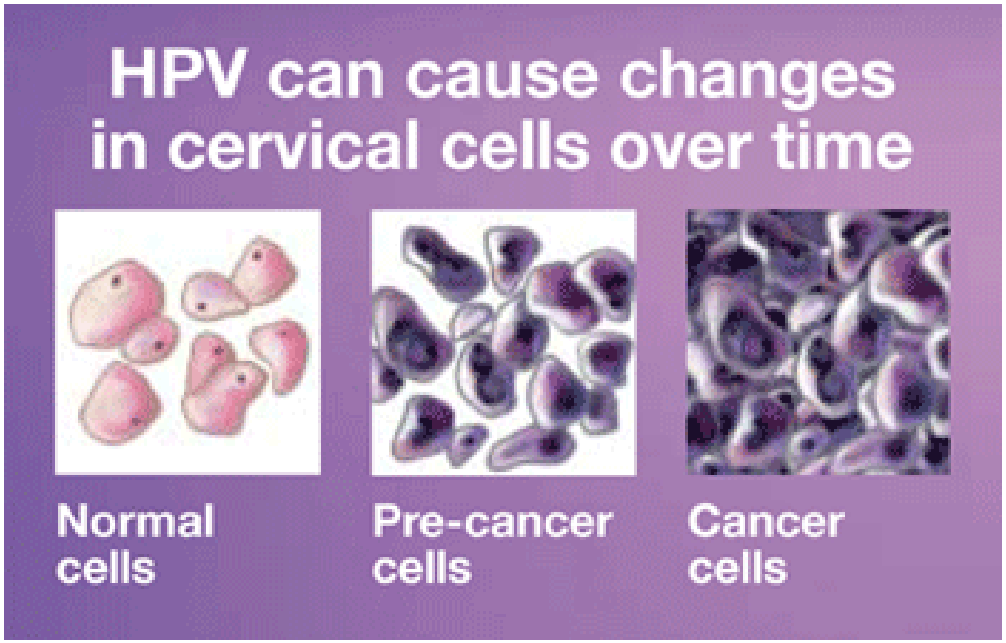


The evolution of cancer following infection takes many years.

Table 1: High-risk and low-risk HPV types	
High-risk (oncogenic or cancer-associated) types	**Low-risk (non-oncogenic) types**
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 82	6, 11, 40, 42, 43, 44, 54, 61, 72, 73, 81
HPV-16 is responsible for ~60% of cervical cancer, HPV-18 for ~10%, HPV-45 and HPV-31 for ~4% each. HPV-33, HPV-52, and HPV-58 together account for ~2% of cervical cancer	These cause benign or low grade lesions and genital warts

How HPV initiates oncogenesis

Genomic integration is not necessary for virus replication, but may be essential for oncogenesis. When the circular viral genome integrates into the host chromosome it disrupts the E2 gene. The E2 protein normally controls the expression of E6 and E7 (prevents excessive expression of these genes).
Uncontrolled expression of E6 and E7 makes the cell vulnerable by respectively inactivating the anti-oncogenes p53 and retinoblastoma protein (pRB). These anti-oncogenes work together to protect the cell from DNA damage:
p53 (inactivated by E6) is a transcription factor which controls DNA repair and the cellular response to injury. It initiates apoptosis in a cell that is too badly damaged.
The retinoblastoma protein (inactivated by viral E7) halts DNA replication during the G1 phase of the cell cycle to allow repair of damaged DNA.
Thus HPV-infected cells fail to repair damaged DNA, accumulate mutations and do not undergo apoptosis. This makes them vulnerable to malignant transformation.



Detection of pre-malignant lesions

Cytological screening tests

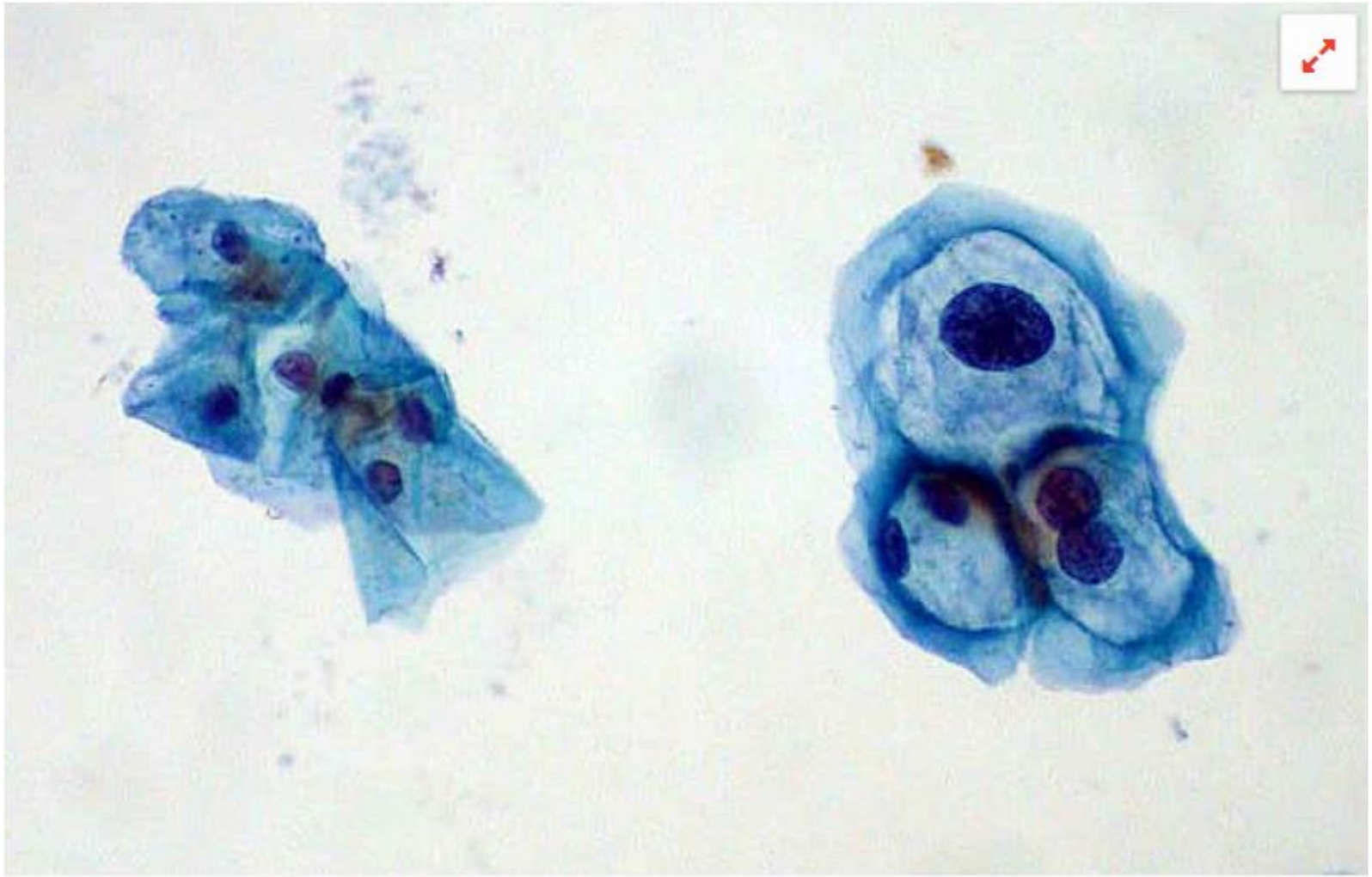
Pap smear

The most widely used screening test for cervical cancer is the Pap smear. Cells collected directly from the cervix with a swab or cytobrush are smeared onto a glass slide, fixed and stained and examined microscopically for evidence of cellular atypia. Cells are scored as normal, low grade squamous intra-epithelial lesion (LSIL), high grade squamous intra-epithelial lesion (HSIL) or atypical cells of unknown significance (ASCUS). The main problems with using this as a screening test is that it is labour intensive and requires skilled cytologist ton assess the slides. Also a reliable result requires an adequately collected sample.

HPV DNA tests

Because cancer of the cervix only develops in patients persistently infected with high risk HPV types, the detection of high risk HPV DNA in cervical cells is a sensitive way to identify patients at risk for cervical cancer. However, although these tests are highly sensitive and specific, they cannot distinguish between patients with a transient or persistent HPV infection. It has a high negative predictive value for carcinoma of the cervix (i.e. if negative, the patient is very unlikely to be evolving carcinoma of the cervix). This test is a useful adjunct to cytology for:

1. older women (if positive, likely to be persistently infected with high risk HPV type)
2. For determining the significance of ASCUS
3. Identifying women who need more regular follow up.



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Pap Smear.
The clump on the left are normal cervical cells. The ones on the right are infected with human papillomavirus, a frequent cause of cervical cancer.

HPV vaccines

Protective immunity to HPV is directed to the major capsid (L1) protein. Immunity is type specific and there is little cross protection with other HPV types. After natural infection only 50-70% people develop detectable antibody and this takes many months to develop (as virus evasion delays onset and magnitude of specific immune response). This accounts for why people can be repeatedly infected with HPV throughout their sexually active life.

A major breakthrough has been the development of subunit HPV vaccines, based on the L1 protein of specific HPV types. The vaccines consist of recombinant L1 protein. The L1 protein self assembles into virus-like particles (VLPs) which are highly immunogenic. A course of 3 doses induces high levels of type specific antibodies in vaccine recipients (much higher than is induced by natural HPV infection). To prevent infection, vaccine needs to be administered before onset of sexual activity (before first exposure to HPV), currently advised for pre-pubertal girls.

2 vaccines have been licensed so far:
1. Cervarix contains VLPs derived from HPV16 and 18. Together these high risk HPV types account for 70% of cervical cancers.
2. Guardasil contains VLPs from HPV16 and 18 as well as for 6 and 11 (the last 2 are the major cause of genital warts.)

Clinical trials of these two vaccines have shown both vaccines to be highly effective at preventing type specific HPV infection in vaccine recipients. Many countries have added this vaccine to their national immunisation programmes (targeting pre-pubertal girls), but they are still very expensive and in South Africa are not available to the public sector.

Contents lists available at ScienceDirect

Pathology – Research and Practice

journal homepage: www.elsevier.com/locate/prp

Original article

Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an instutional experience from Turkey and review of literature

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Table 1
Presence of HPV-DNA in the esophageal SCC and adenocarcinoma and the control group.

Diagnosis	HPV		Total
	(−)	(+)	
SCC	30 (90.9%)	3 (9.1%)	33
Adenocarcinoma	17 (89.5%)	2 (10.5%)	19
Control group	8 (100%)	0 (0%)	8
Total	55	5	60

Table 2
HPV positive cases and HPV types.

Case no	Tumor type	HPV type
H21	Squamous cell carcinoma	Type 39
H22	Squamous cell carcinoma	Type 39
H54	Squamous cell carcinoma	Not available
H68	Adenocarcioma	Type 39
H41	Adenocarcioma	Type 16

Reference; Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an instutional experience from Turkey and review of literature, Pathol. – Res. Pract (2015), http://dx.doi.org/10.1016/j.prp.2015.10.007

Results of real-time HPV PCR in the parafin block (pathological) samples

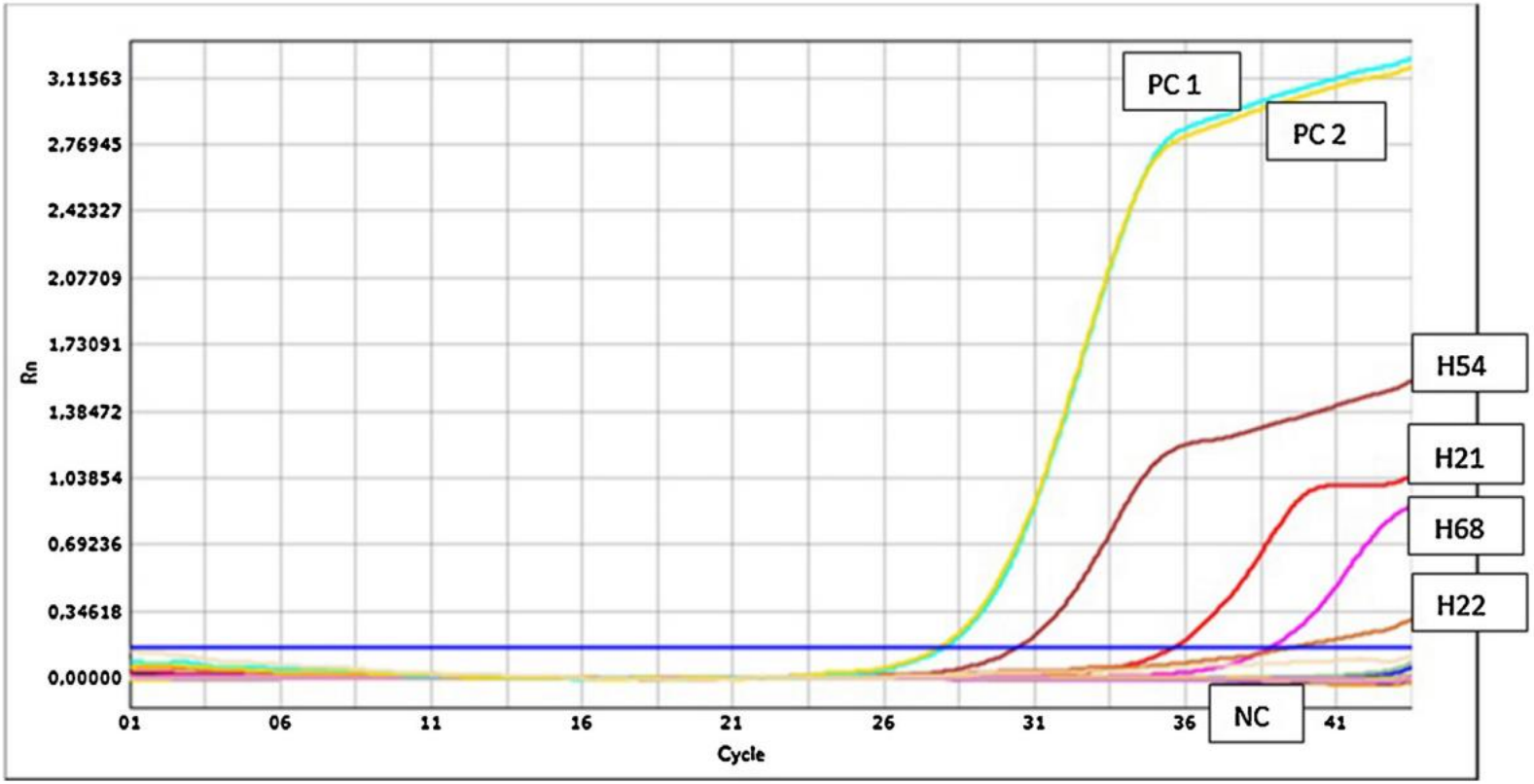

Fig. 1. HPV real-time PCR analysis Footnotes: PC; positive control, NC; negative control, H; HPV DNA-positive patient sample.

Reference; Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an instutional experience from Turkey and review of literature, Pathol. – Res. Pract (2015), http://dx.doi.org/10.1016/j.prp.2015.10.007