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Human Papillomavirus: Detection and prevention of infection

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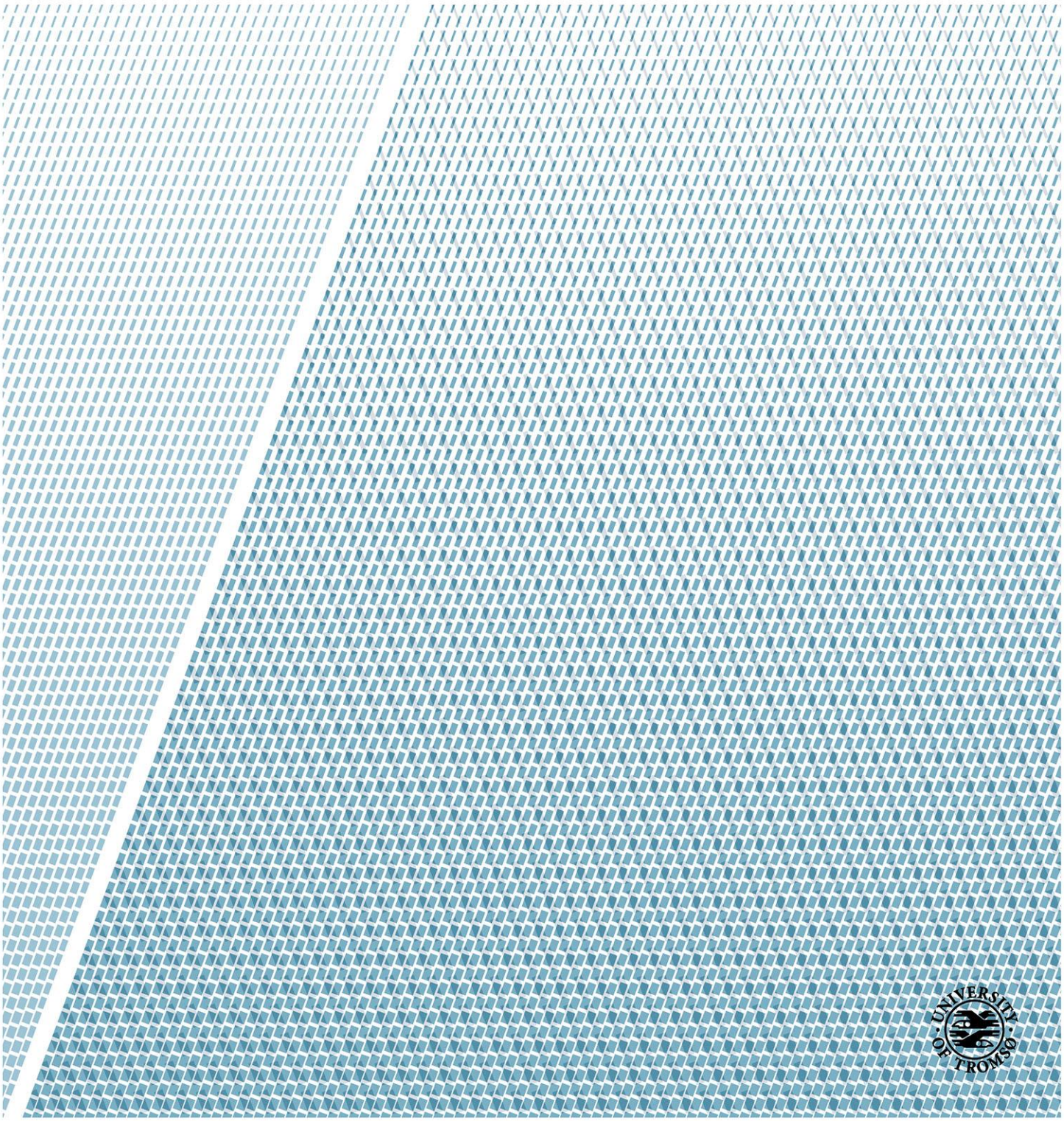
Master thesis in odontology, May 2017



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Acknowledgement

I am truly grateful for all the help and instructions from my supervisor Mohammed Al-Haroni. A special thanks to him.

Human papillomaviruses and cancer have been a big topic in social media lately, and this is an engaging and important topic for all of us. We can prevent infections, and we can use different tests to identify cell changes before they become cancerous. With this, I will recommend all women to take cervical cell samples every third year, and all those young who get vaccines for free to accept the offer.

Abstract

Human papillomaviruses (HPVs) are composed of a big group of over hundred related viruses. Some of them can cause warts, and some can in worst case lead to cancer. High-risk HPVs can cause several types of cancer such as: cervical cancer, anal cancer, oropharyngeal cancers (cancers of the middle part of throat, including the soft palate, the base of the tongue, and the tonsils), vaginal cancer, vulvar cancer and penile cancer.

The high-risk HPVs cause approximately 5 % of all cancers worldwide. It is well known that oral cancer is related to tobacco and alcohol use. On the other hand, HPVs seem to be linked to many of the cases of oral cancer. In Norway 57 % of oropharyngeal cancers are related to HPV infections. Nearly 70 % of all humans will be infected by HPV during life. Most of HPV infections go away on their own, but some infections persist and can cause cellular changes in the infected tissues.

The identification of HPV nowadays relies on molecular biology techniques. This is because it cannot be propagated in tissue cultures. HPV has a well-known physical structure and an organization of genes, making the tests of choice for detecting HPV from clinical specimens based on nucleic acid probe technology. The detection methods can be divided into target amplification methods and signal amplification methods. Polymerase chain reaction (PCR) is the most commonly used tool in the detection of HPVs DNA.

Since 2009, a vaccine (Gardasil) against 4 types of HPVs has been offered to 12-year-old females in Norway. The main purpose of this vaccine was to prevent the occurrence of cervical cancer among Norwegian females. It is estimated that 100 % of all cervical cancers are related to HPV infections. About 10 000 Norwegian females get diagnosed with mild cervical cell changes every year. About 300 Norwegian females are diagnosed with cervical cancer each year, and every year about 70 Norwegian females die because of cervical cancer. HPV 16 and 18 are considered the main cause of about 70 % of cervical cancer, and HPV 6 and 11 are found in cases mostly related to genital warts. The HPV vaccine is now also offered to Norwegian females born between year 1991 and 1996, 20-25 years of age. Some studies have suggested that the HPV vaccine can protect against HPV infections caused by oral transmission of the virus.

1. Introduction

1.1 Introduction: Human papillomaviruses

Human papillomaviruses (HPVs) are composed of a big group of over hundred related viruses (1). Some of them can cause warts, and some can in worst case lead to cancer. They can be spread through sexual and non-sexual contact, depending on the virus. About 40 HPV types can transmit through direct sexual contact, from the skin and mucous membranes of the infected people to the skin and mucous membranes of their partners (2). Most of HPV infections go away on their own, without any signs or symptoms. This means also that infected people can unknowingly pass HPV to their sexual partners (3). Nearly 70 % of all humans will be infected by HPV during life (4). Some people get persistent infections with HPV, and that can cause cellular changes in the infected tissues (5).

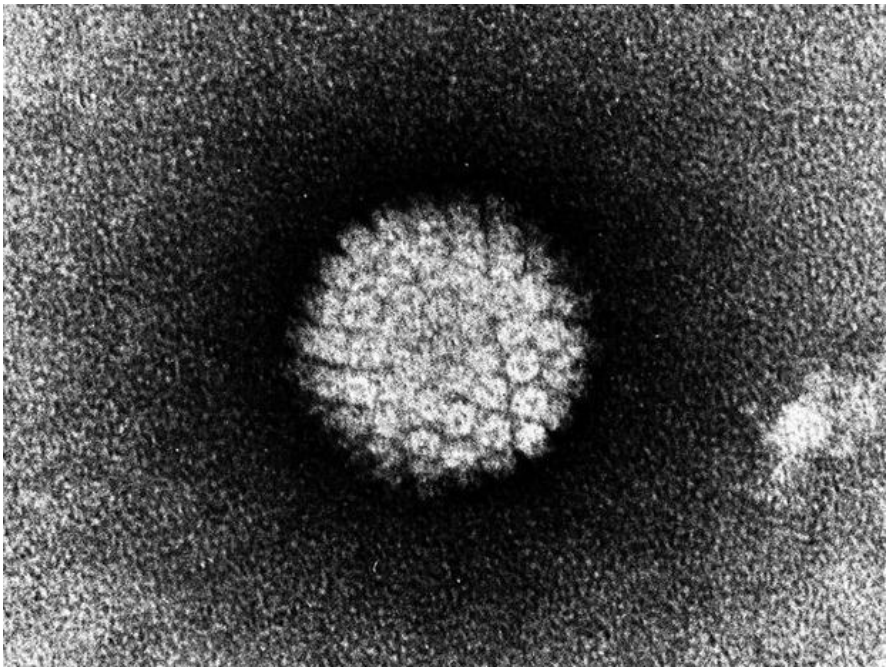

Figure 1. Human Papillomavirus shown under an electron microscope(6)

1.2 The link between HPV and cancer

The Human papillomavirus was first discovered in 1956, by a group of scientists. At that time, it was difficult to investigate the virus and study the individual viral genes, because of the lack of cloning techniques (7).

There had for a long time been seen a potential link between a viral infection and cervical cancer. When scientists did their search, it was important to compare the lifestyle of women with cervical cancer and women without the disease. The observation was surprising. They found that prostitutes had a high risk of getting the disease, married women had a moderate risk, and nuns were mostly spared from it (8). The risk of cervical cancer correlated with the number of sexual partners, which suggested that a sexual transmitted agent played an important role.

The search of the viral agent causing cervical cancer lasted many years. There were many diseases and viral infections that made a false lead in finding the cause. But in the 1980s the German virologist Harald Zur Hausen did a big discovery (7). Harald Zur Hausen had heard about the papillomavirus in rabbits, a research done by Richard Shope from the 1930s (8). Richard Shope found that rabbits with horns growing out of their body had an infection. He found that they were infected with a type of papillomavirus, that caused warts (horns) and cancer in rabbits (9). Harald Zur Hausen began to search for a similar virus that could infect humans. He then found HPV 6 in genital warts (10). But after more research it was clear that this virus was rarely found in cases with cancer. It was neither the same virus causing warts on hands or feet. This made him think of the possibility that there could be several types of HPVs, causing different symptoms, virulence, and malignancy. Harald Zur Hausen managed to clone DNA from cervical cancer tissues. Sequence analysis of the DNA revealed new strains of HPV, HPV 16 and HPV 18 (11, 12). After analyzing many women with cervical cancer, they found that 70 % of them had cancer cells containing these two types of HPV. By the end of the 20th century, over 100 types of HPV were identified (7). And today we know that the prevalence of HPV DNA in cervical cancer approaches nearly 100 % (13). In 2008, Harald Zur Hausen received the Nobel Prize for his discovery of human papilloma viruses causing cervical cancer (8).

1.3 Low-risk HPVs and high-risk HPVs

We can divide HPVs into low-risk HPVs and high-risk HPVs. Low-risk HPVs are the types that can cause warts(14). HPV 6 and HPV 11 are found in 90% of genital warts. These viruses can transmit by direct sexual contact like vaginal-, anal-, and oral sex, and when skin or mucous membranes are in contact (5). These viruses can be transmitted before any sexual intercourse, and even with the use of prevention measures like condoms. This is because infected skin can be in contact with healthy skin, and transmit the virus. The warts can arise on the genitals, around the anus, mouth, or throat. These two viruses can also lead to a rare condition called recurrent respiratory papillomatosis (RRP)(15). This disease causes symptoms in the upper respiratory airway. Warty growths, benign tumors, may lead to significant airway destruction or change of voice. Kids age 5 years or younger are likely to have get infected during the period of their childbirth, while adults seem to get it from sexual transmission(16).

On the other hand, high-risk HPVs can cause several types of cancer such as: cervical cancer, anal cancer, oropharyngeal cancers (cancers of the middle part of throat, including the soft palate, the base of the tongue, and the tonsils), vaginal cancer, vulvar cancer and penile cancer (5). The term high-risk is based on whether they put a person at risk of developing cancer or not(14). These viruses can transmit by direct sexual contact like vaginal-, anal-, and oral sex, and when skin or mucous membranes are in contact. HPV 16 and HPV 18 are responsible for most HPV-caused cancers. It is estimated that the high-risk HPVs cause approximately 5 % of all cancers worldwide (5). There are several factors that can contribute in the persisting of a high-risk infection of HPV, and the developing of cancer. The factors that increase the risk are smoking, weakened immune system, early sexual debut, multiple sexual partners, and chronic inflammation(17).

1.4 Structure of the Human papillomavirus

HPVs are small non-enveloped DNA viruses (19). The circular, double stranded viral genome consists of approximately 8 kb. The viruses have a diameter of 52-55 nm (20). The genome encodes for six early proteins responsible for virus replication and two late proteins, L1 and L2, which are the viral structural proteins. Papillomaviruses replicate and assemble exclusively in the nucleus (21).

The Viruses infect the keratinocytes in the basal layers of stratified squamous epithelium. The replication and expression of the viral gene is proceeded in a controlled fashion, and are regulated by keratinocyte differentiation. This process is not fully understood, but there is a general agreement about the six regulatory proteins (E1, E2, E4, E5, E6 and E7) and two viral structural capsid proteins (L1 and L2) (16). E1 and E2 are involved in the DNA replication of the viral DNA and the regulation of the early transcription. They act as factors that recognize the origin of replication, where E2 also is the main regulator of the viral gene transcription (20). E4 associates with cytokeratin filament collapse, when expressed in a productive infection. The E4 protein is believed to be involved in the late stages of life cycle of the virus, and continues to be expressed in the terminally differentiated keratinocytes (19, 20). E5 expression induces cell immortalization and transformation. E5 may function during both the early and the late face(19, 20). Two of them, E6 and E7, are viral oncoproteins. They inactivate p53 and pRb, which are cellular tumor suppressor proteins (22, 23). L1 and L2 encapsidate the viral genomes to form progeny virions in the nucleus. The shed virus can then initiate a new infection (23).

Table 1. Function of the regulatory- and capsid proteins (61,62)

GENE	FUNCTION	HPV16-SEQUENCE	HPV18-SEQUENCE
L1	MAJOR CAPSID PROTEIN	4775-6292	5430-7136
L2	MINOR CAPSID PROTEIN	3373-4794	4244-5632
E1	DNA REPLICATION, RECOGNIZE ORIGIN	1-1950	914-2887
E2	MAIN REGULATOR OF THE VIRAL GENE TRANSCRIPTION	1892-2989	2817-3914
E4	CYTOKERATIN FILAMENT COLLAPSE, VIRION RELEASE	E1^E4: 1-2756	3418-3684
E5	CELL IMMORTALIZATION AND TRANSFORMATION	2986-3237	396-4157
56	VIRAL ONCOPROTEIN	7125-7601	105-581
E7	VIRAL ONCOPROTEIN	7604-7900	590-907

Papillomaviruses are epitheliotropic, and they establish productive infections only within stratified epithelia of the skin, oral cavity, and the anogenital tract (19). The viral life cycle is linked to the differentiation of the infected epithelial cell. The progression of untreated lesions to invasive cancer is associated with the integration of the HPV genome into the host chromosomes, with associated loss or

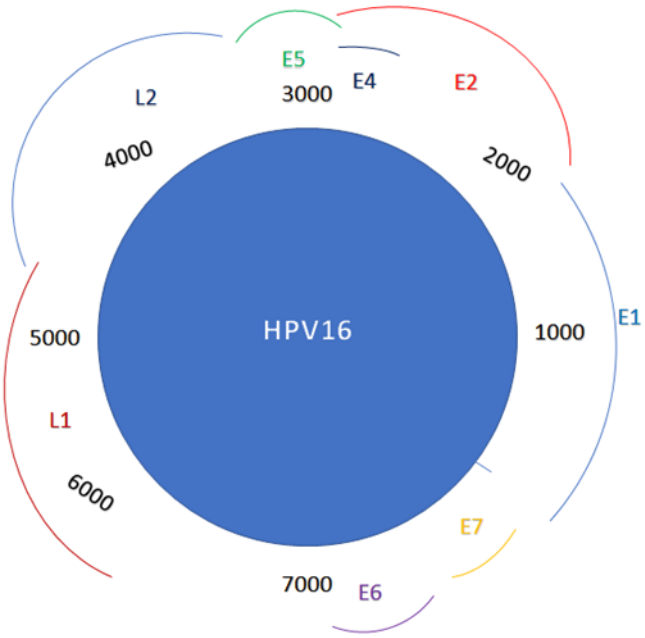

Figure 2. HPV 16

disruption of E2, and upregulation of E6 and E7 oncogene expression. E6 and E7 from low-risk HPVs, inactivate cellular p53 and pRb tumor suppressor proteins less efficiently than E6 and E7 from high-risk HPVs (21).

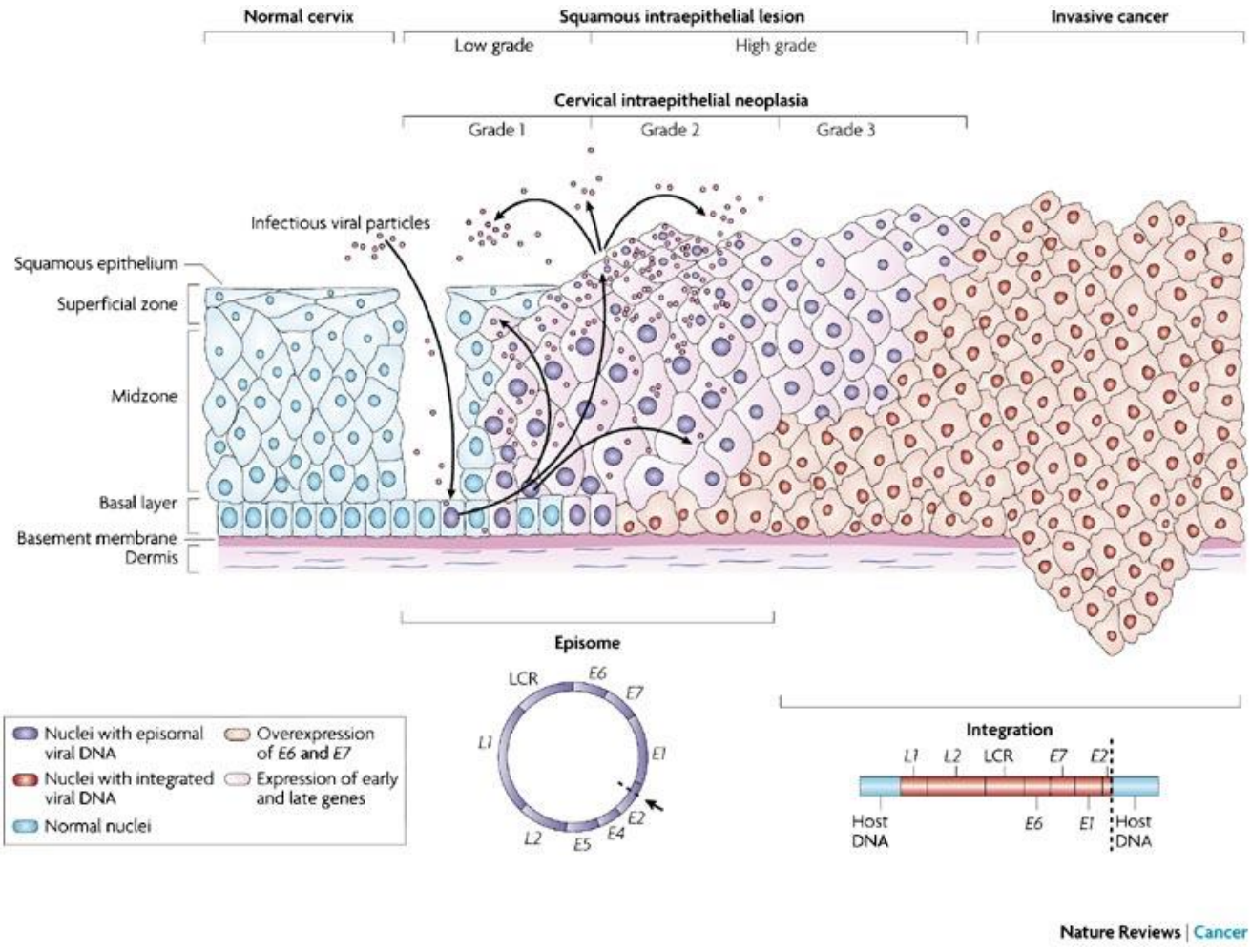

Figure 3. HPV mediated progression to cervical cancer(23)

1.5 Comparison of HPV 16 and HPV 18 genomes

When comparing HPV 16 and HPV 18 we can see that they do not have any identical genome, but parts of it has somewhat similar sequences. The red areas in the table below are the alignment scores that show the parts with most similarities between the two of them.

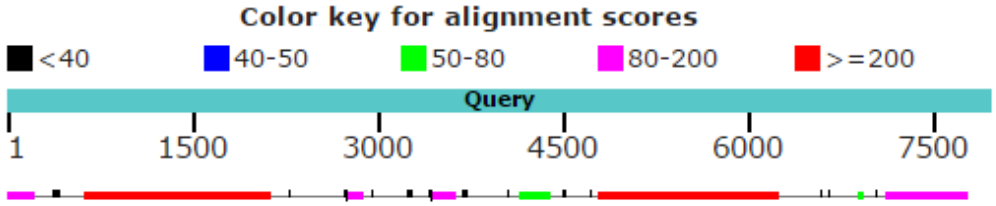

Figure 4. Comparison of HPV 16 and HPV 18 (64)

The vaccines have been made with virus-like particles (VLPs) of the recombinant major capsid (L1). When we compare L1 of HPV 16 and HPV 18 in the figure beneath, we see that the alignment score is high, and that is why they can use particles resembling this area in the vaccine.

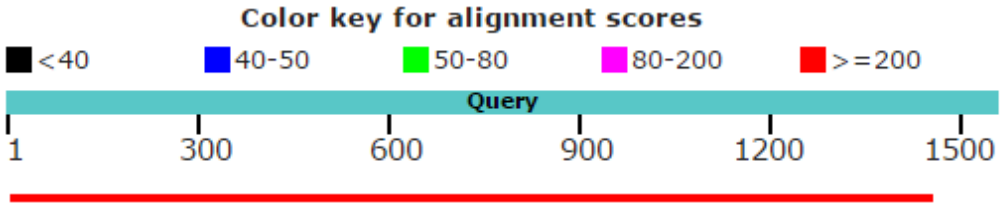

Figure 5. Comparison of L1 in HPV 16 and HPV 18 (64)

2. Methods for detection of HPVs

The identification of HPV nowadays relies on molecular biology techniques. This is because it cannot be propagated in tissue cultures. HPV has a well-known physical structure and an organization of genes, making the tests of choice for detecting HPV from clinical specimens based on nucleic acid probe technology(23). The detection methods can be divided into target amplification methods and signal amplification methods(24). Here are some examples.

2.1 Target amplification methods

a) Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is the most commonly used tool in the detection of HPVs DNA. In this detection system, a spectrum of HPV types is amplified by consensus primers, followed by detection with type-specific probes. These techniques are specific, highly sensitive, and widely used(25). The thermostable DNA polymerase recognizes and extends a pair of oligonucleotide primers that flank the region of interest. PCR can theoretically produce one billion copies from a single double-stranded DNA molecule after 30 cycles of amplification(26). Typically, PCR procedures for HPV detection use primers targeted to the viral capsid L1 gene, which can detect numerous HPV types. Commonly used L1 consensus primer sets include PGMY09/11, GP5+/6+, and SPF10, along with a few proprietary primers having the ability to identify a large range of HPV types with 1 amplification(27-31).

Table 2. Examples of primers used to identify HPV16 and HPV18(63)

TYPE	PRIMER	SEQUENCE	POSITION
HPV16	**PR1**	**5'- TCA AAA GCC ACT GTG TCC TGA- 3'**	**421-440**
HPV16	**PR2**	**5'- CGT GTT CTT GAT GAT CTG CAA- 3'**	**521-540**
HPV18	**PR1**	**5'- CCG AGC ACG ACA GGA ACG ACT-3'**	**533-555**
HPV18	**PR2**	**5'- TCG TTT TCT TCC TCT GAG TCG CTT- 3'**	**682-705**

Typing of PCR products was traditionally done by means of Southern blotting and in-situ hybridization with type-specific oligonucleotides(24).

b) Southern blot

For HPV genome analysis, hybridization in solid phase, such as Southern blot for DNA, is an excellent procedure that can generate information with quality, but it is time consuming. It requires large amounts of highly purified nucleic acids and well preserved, full-size molecules. Southern blotting is the transfer of DNA fragments from an electrophoresis gel to a membrane support, resulting in immobilization of the DNA fragments, so the membrane carries a semi-permanent reproduction of the banding pattern of the gel. After immobilization, the DNA can be subjected to hybridization analysis, enabling bands with sequence similarity to a labeled probe to be identified(32-36).

c) In-situ Hybridization

In-Situ Hybridization (ISH) is a technique that allows for precise localization of a specific segment of nucleic acid within a histologic section. The underlying basis of ISH is that nucleic acids, if preserved adequately within a histologic specimen, can be detected through the application of a complementary strand of nucleic acid to which a reporter molecule is attached(37). Like Southern blot, this technique needs large amount of purified DNA, and is very time consuming. A disability is that this technique has a low sensitivity (35, 38-40).

d) Type-specific PCR

It is possible to find specific types of HPV, by designing primers. With real-time PCR assay, we can quantify the HPV in the specimen. Cervical smear can be analyzed by real-time PCR, and the amount of high risk HPV is predictive for the presence or development of high-grade cervical lesions(24). Smear tests (pap test) are used for cervical screening. Samples of cells from the cervix can be collected and examined for early cell changes. With this test, it is possible to detect cell changes before they are becoming cancerous(33, 41-43).

e) mRNA amplification

Recently it has been shown that viral mRNA can be detected, and therefore a method for finding HPV. The transcripts that are relevant in the search, are the viral oncoproteins E6 and E7. A hypothesis suggests that viral mRNA from these oncogenes in smear from the cervix have a better positive predictive value for high-grade cervical lesions than the presence of viral DNA. The explanation is that E6/E7 mRNA represent an active infection with cell-transforming potential, whereas viral DNA can be present in clinically irrelevant conditions as well. Detection of mRNA can be done by reverse-transcriptase PCR or nucleic acid sequence-based amplifications (NASBA)(24, 34, 44-46).

2.2 Signal amplification methods

a) Liquid-phase signal amplification techniques

An example of this type of detection is Hybrid Capture 2 (HC2). This high-risk HPV DNA test is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. This method uses a cocktail of full-length RNA probes representing the high-risk HPV searched for(24). The resultant RNA: DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for RNA: DNA hybrids. Immobilized hybrids are then reacted with alkaline phosphatase conjugated antibodies specific for the RNA: DNA hybrids, and detected with a chemiluminescent substrate. Several alkaline phosphatase molecules are conjugated to each antibody. Multiple conjugated antibodies bind to each captured hybrid resulting in substantial signal amplification. As the substrate is cleaved by the bound alkaline phosphatase, light is emitted that is measured as relative light units (RLUs) on a luminometer. The intensity of the light emitted denotes the presence or absence of target DNA in the specimen(47)

2.3 Clinical specimen

Detection with PCR can generally use all kinds of clinical specimen, if the DNAs contained within are not heavily degraded, cross-linked, or with presence of PCR-inhibiting factors(24). It is possible to use saliva, paraffin-fixed/paraffin embedded tissue, and smears to mention some examples.

3. HPV related to cancer

3.1 HPV: Oral cancer

Oral cancer is classified as head and neck cancer and head and neck squamous cell carcinoma (HNSCC)(48). HPV 16 is the human papilloma virus which is mostly associated with malign lesions in the oral cavity(49). HPV- related cancers have a better prognosis than HPV-negative tumors in overall survival rates and clinical response to treatment. There have been found 24 types of HPV associates with benign lesions, and 12 different types associated with malign lesions. About 99 % of HPV infections in HNSCC are related to the high-risk types HPV-16, HPV-18, HPV-31, and HPV-33. Most of the infections are related to HPV-16, and HPV-33 second most with up to 10% of the cases (48).

3.2 HPV: Presence in Norway

Nearly 70 % of all humans will be infected by HPV during life (4). It is well known that oral cancer is related to tobacco and alcohol use. On the other hand, HPVs seem to be linked to many of the cases of oral cancer(50). In Norway 57 % of oropharyngeal cancers are related to HPV infections (statistics from 2014) (4).

Approximately 600 causes of cancer in Norway can be related to a HPV infection. Today it is more infection of these viruses among people than previously reported. This can be related to the changes in sexual behavior, and an increase in the number of sexual partners. It is important to remember that only one sexual partner is enough to be in the risk of getting an infection (4).

3.3 HPV, cervical cell changes, and cancer in Norway

HPV is very relevant in the relation to cervical cancer in women. It is estimated that 100 % of all cervical cancers are related to HPV infections. About 10 % of all Norwegian women will get genital warts before the age of 45. About 10 000 Norwegian females get diagnosed with mild cervical cell changes every year. 3000 Norwegian women get severe cell changes every year, that lead to removal of cells in the cervix. This will raise the risk of spontaneous abortion and early births. About 300 Norwegian females are diagnosed with cervical cancer each year, and every year about 70 Norwegian females die because of cervical cancer. There are also 300 other cases of cancer related to HPV, in both men and woman each year. These statistics are obtained from analysis of cases in 2014(4).

3.4 HPV related to cancer in Norway

In year 2014, 338 Norwegian women were diagnosed with cervical cancer. 100 % of these cases were related to a HPV infection. A total of 61 Norwegian women and 25 Norwegian men were the same year diagnosed with anal cancer. Approximately, 90 % of these cases were related to an infection of HPV. In addition, 19 Norwegian women were in 2014 diagnosed with vaginal cancer, 81 % of these women had an infection of HPV. 39 Norwegian women and 133 Norwegian men were diagnosed with oropharyngeal cancer, and 57 % of these cases were seen in relation to a HPV infection. On the other hand, 54 Norwegian men were diagnosed with penile cancer, 47% were related to an infection of HPV. Furthermore, 89 Norwegian women were in 2014 diagnosed with vulva cancer, 29 % of the cases were related to HPV (4).

4. Vaccination

4.1 Vaccination in Norway

Today we have three different vaccines against HPVs. The vaccines consist of non-living material, which resemble the surface of the Human papilloma virus. The virus-like particles comprising the major capsid protein L1 of the high-risk HPV-16 and HPV-18(51). The vaccines cannot cause a HPV infection(52).

In Norway 12-year-old females can get the vaccine through the vaccination program. The vaccine has been available in the program since 2009(53). This vaccination is done to prevent cervical cancer. HPV is also related to other cancers, so the Government of Norway suggest to offer 12-year-old males the vaccine as well(54).

The vaccine cannot remove a persistent infection, so it is advisable to vaccinate before sexual debut. The vaccine protects against several types of HPV, so even if you have had an infection or not, it can prevent you from getting a new infection or an infection from one of the other types of HPV. Therefore, the Government of Norway offers girls born after 1991 the vaccine for free (November, 2016)(53).

4.2 Vaccination of young males

The suggestion to offer young males the HPV vaccine is a step in the right direction. HPVs are mostly something people relate to women and cervical cancer, but new knowledge show us that HPVs are related to so many other types of cancer. It is important to prevent both sex from getting infected from these viruses, if we want to stop the progression of HPV-induced cell changes. The high-risk virus can transmit by all sorts of sexual contact, and we live in a society where sexual relations are between individuals with opposite gender or with the same gender. That is one of the good arguments why vaccination of only women is not enough. Men also need to be protected from cancers associated with HPV(55-57).

4.3 Contents and protection

All three vaccines available protect against HPV16 and HPV18, which cause 70% of all cervical cancers. Cervarix is the commercial name of the vaccine which only protect against these two types of HPV. All females between 16 and 25 years of age are offered this vaccine. Females younger than 16 years, get the vaccine called Gardasil, which protect against HPV16 and HPV18, together with HPV6 and HPV11. The last two HPVs can cause 90 % of all genital warts, also called condylomas(53). The last vaccine is not available in Norway yet, but it protects against nine types of HPV; HPV6, HPV11, HPV16, HPV18, HPV31, HPV33, HPV45, HPV52, HPV58. Beside HPV6 and HPV11, these are high-risk HPVs which can cause cellular changes in the infected tissues and lead to cancer(52).

Figure 6. Cervarix and Gardasil

| Gardasil contains virus-like particles (VLPs) of the recombinant major capsid (L1) protein of HPV types 6, 11, 16, and 18. These are the active substances in the vaccine. The recombinant proteins forming the VLPs are produced by separate fermentation in recombinant Saccharomyces cerevisiae. The viral proteins are manufactured in yeast cells. Once released from yeast cells, the VLPs are purified. VLPs of each type are adsorbed on amorphous aluminium hydroxyphosphate sulfate adjuvant. The formulation also includes sodium chloride, L-histidine, polysorbate 80, sodium borate, and water for injection. The final product is presented as a sterile suspension, either in a single-dose vial or in a prefilled syringe for intramuscular injection(58). Gardasil is given in three doses, month 0, 2, and 6.

The HPV vaccine gives a 90 % protection against the HPVs it is supposed to protect against. It has been tested for a period now, and we know that the vaccine protects individuals for at least a decade. Some believe it can protect a person throughout life. But it has not been tested long enough to know if people will need to get a booster-dose to make it last longer or not(52).

Even though vaccination protect against some HPVs it will still be important for women to take cell samples from the cervix, every third year after the age of 25. This is because the vaccine does not protect against all HPVs that are high-risk(52).

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6. Appendix I

6.1 Human papillomavirus type 16, complete genome

ORIGIN

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781 tatttacaca ttcaaagttt agcatgttca tggggaatgg ttgtgttact attagtaaga
841 tataaatgtg gaaaaaatag agaaacaatt gaaaaattgc tgtctaaact attatgtgtg
901 tctccaatgt gtatgatgat agagcctcca aaattgcgta gtacagcagc agcattatat
961 tggtataaaa caggtatatc aaatattagt gaagtgtatg gagacacgcc agaatggata
1021 caaagacaaa cagtattaca acatagtttt aatgattgta catttgaatt atcacagatg
1081 gtacaatggg cctacgataa tgacatagta gacgatagtg aaattgcata taaatatgca
1141 caattggcag acactaatag taatgcaagt gcctttctaa aaagtaattc acaggcaaaa
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1561 ttagatgatg ctacagtgcc ctgttggaac tacatagatg acaatttaag aaatgcattg
1621 gatggaaatt tagtttctat ggatgtaaag catagaccat tggtacaact aaaatgccct

1681 ccattattaa ttacatctaa cattaatgct ggtacagatt ctaggtggcc ttatttacat
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7741 accggacaga gcccattaca atattgtaac cttttgttgc aagtgtgact ctacgcttcg
7801 gttgtgcgta caaagcacac acgtagacat tcgtactttg gaagacctgt taatgggcac
7861 actaggaatt gtgtgcccca tctgttctca gaaaccataa tctacc //

6.2 Human papillomavirus type 18, complete genome

ORIGIN

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61 gaaaacggtg tatataaaag atgtgagaaa cacaccacaa tactatggcg cgctttgagg
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