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=== REVIEW ===

Human Papilloma Virus infection and cervical cancer in Romania

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SUMMARY. Infection with human papillomaviruses (HPV) is a major public health burden worldwide and is associated with a variety of epithelial lesions, including benign warts and several types of anogenital tumors, particularly cervical carcinoma.HPV can be grouped into cutaneous types and mucosal types based on their preferred tissue tropism. Cutaneous types are typically found in the general population and cause common warts. Mucosal HPV is further classified into high-risk and low-risk types, based on their association with cervical cancer. The most common low-risk types are HPV 6 and 11, detected most often in benign genital warts. HPV 16, 18, 31, and 45 are predominant types found in cervical squamous cell carcinoma. HPV 16 is the most prevalent type in cervical cancer (55%), followed by HPV 18 and HPV 45. Epidemiological evidence has convincingly demonstrated that infection with HPV is the greatest risk factor, its role in the progression of the precursor lesions to cervical cancer is well established. HPV is exclusively epitheliotropic, and their replication is linked to the differentiation process of the host cells. Normal squamous epithelial cells grow as stratified epithelium, with those in the basal layers dividing as stem cells of transient amplifying cells. After division, one of the daughter cells migrates upward and begins to undergo terminal differentiation while the other remains in the basal layer as a slow-cycling, self-renewing population. Productive papillomavirus infection begins when infectious virions gain access to cells of the basal layer, probably through micro-wounds. The viral genome is maintained in these cells at low copy number. These infected cells from the reservoir for the development of a productive wart. Early HPV genes E1 and E2 support viral DNA replication and its segregation such that the infected cells can be maintained in the lesion for a long period. As infected daughter cells migrate towards the epithelial surface, viral late gene products are produced to initiate the vegetative

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phase of the HPV life cycle, resulting in the high-level amplification of the viral genome. In the outer layers of the epithelium viral DNA is packaged into capsids and progeny virions are released to reinitiate infection. Given the worldwide burden of HPV infection (anogenital warts and neoplasia of several sites), prevention of infection could provide relief from an important public health threat. With the introduction of cervical screening in developed countries, the number of deaths from cervical cancer has declined dramatically, but in developing countries it still remains the number one of female cancer.

Keywords: cervix cancer, Human papilloma virus, koilocyte

The Human papillomavirus (HPV) infection is now a well-established cause of cervical cancer and there is growing evidence of HPV being a relevant factor in other anogenital cancers (anus, vulva, vagina and penis) and head and neck cancers (Anic and Giuliano, 2012, Deng *et al*., 2015). HPV types 16 and 18 are responsible for about 70% of all cervical cancer cases worldwide (Gross, 2014).

Romania has a population of 9.54 million women aged 15 years and older who are at risk of developing cervical cancer. Current estimates (*http://www.hpvcentre.net/statistics/reports/ROU.pdf, 2015*) indicate that every year 4343 women are diagnosed with cervical cancer and 1909 die of the disease.

AIDS, the plague of our age, demands approximately 1200 in Romania yearly. It is not known widely, but cervix cancer due to infection with human papilloma virus (HPV) demands more than 2000 in Romania every year (*http://www.hpvcentre.net/statistics/reports/ROU.pdf, 2015*). According to the survey of the WHO more than 4300 women are infected with HPV virus in Romania yearly. In our country HPV is the third most frequent cancer among women and the most frequent cancer type affecting women between ages 15 and 44. In Romania 9.54 million women are above age 15, they have the highest risk of cervical cancer.

This virus has been known in medicine research for 100 years, and earlier it was thought that it caused warts and skin growths in the epithelium. Mutations of the virus have been found in South-American Indian tribes too, and it justifies that the virus appeared in the early age of human race. HPV infection can incubate for a long time without causing any symptoms, or it can cause epithelial lesions in sexual organs and it can cause warts on different parts of body. The correlation between HPV and cervical cancer was shown by Harald zur Hausen, German scientist, for which he was awarded the Nobel Prize for medicine in 2008. He started his research in 1980 and his

suspicion was awakened by observations that cervical cancer was higher in prostitutes than in the average population. Harald zur Hausen had found that cervical cancer does not occur without HPV cervical cancer *(http://www.nobelprize.org/nobel_prizes/medicine/laureates/2008/).*

HPV infection occurs frequently all over the world and in certain countries (US states) men are virus carriers in similar rate as women (Anic and Giuliano, 2012). In other countries (mostly in European countries) the majority of virus carriers are women. The virus is transmitted through sexual contacts, by certain body fluids from vagina and rectum, or with mouth contact. Viruses foothold in the mucosa of motioned genitals. The probability of infection is increases with injured mucosa (for instance injuries in wall of vagina). In their lives every second woman goes through HPV, 60 percent of which is high risk infection.

Human papillomavirus (HPV) is well known as the major etiological agent for anogenital cancer. In contrast to cervical cancer, anal cancer is uncommon, but is increasing steadily in the community over the last few decades (Moscicki*et al.,* 2012, Stanley *et al.*, 2012).

Given the worldwide burden of HPV infection (anogenital warts and neoplasia of several sites), prevention of infection could provide relief from an important public health threat. With the introduction of cervical screening in developed countries, the number of deaths from cervical cancer has declined dramatically, but in developing countries it still remains the number one of female cancer.

HPV16 genome organization

HPV belongs to the *Papovaviridae* family, it is dezoxyribovirus. Its genome is formed by 8000 bases, coding proteins E1-E7 and L1-L2 (Fig. 1).

Proteins L1 and L2 pack the DNA of the virus, and participate in forming shell pack (capsid). The E1-E7 proteins ensure the function of virus. Since viruses incorporate in DNA of host cell, it is difficult to identify them. This is why a lot of virus infections are difficult to treat. While they multiply in host rapidly, they gain mutation. The structure of next generation differs, so there is no vaccination to ensure long term protection against these viruses (Hamkar and Delforoush, 2009, Lin *et al.,* 2007).

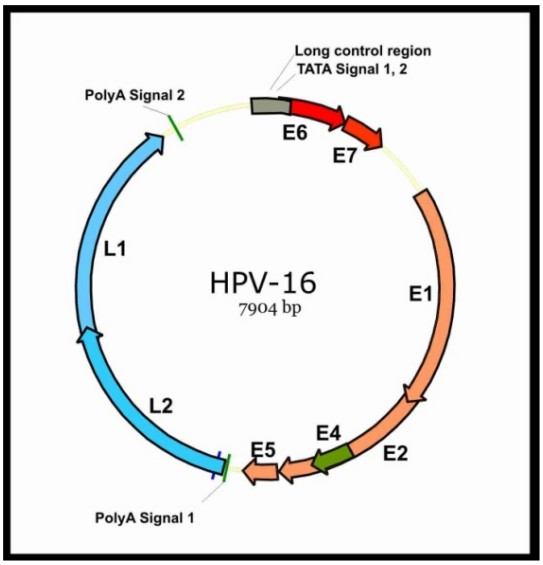

Figure 1. HPV-16 genome organization. The genes have the following functions: L1-L2 capsid protein coding genes having roll in connecting virus to the DNA of the host cell. The vaccination against HPV is connected to this protein too. E1 – it is responsible for replication of DNA, it ensures connection to DNA of host cell. E2 – protein controlling transcription, its inactivity enhances activity of E6-E7. E3 is small in size. It can miss. Its function is not known. E4 is responsible to detaching from host cell. It unseals the cell frame. E5 destabilizes cell membrane. It makes intrusion into the host cell easier. The E5 protein of HPV type 16 inhibits generating MHC1 protein. This protein would indicate modified cell presence for immune cells. In their absence immune cells are not able to recognize the cell with the changed function. E6-E7 hinders cell death. Thus they ensure replication of virus DNA for long and help generating new viruses. They are responsible for generating malignant tumors. They inhibit proteins from hindering tumors generation. As a consequence, the structure and function of host cells change. Instead of eliminating these cells, they divide and generate new mutations, as a consequences new tumor cells come into existence. E8 can be missing from infectious viruses. E8 function is similar to E5 function (wikipedia.org/wiki/Human_ papillomavirus)

HPV subtypes

At least 100 versions of HPV are known. According to epidemiological data (occurrence in precancer states and in cervix cancer) and biogenetical similarity, low, transition and high risk types are distinguished. The HPV viruses can be distinguished where they foothold - anogenetical, oral, mouth mucosal, pharynx, larynx and in skin- (Gross, 2014, Szentirmay *et al.,* 2005).The HPV viruses are marked with Arabic numbers.

The most dangerous, high risk viruses are 16, 18, 31, 33, 35, 39, 45, 51and 52 (Chaturvedi *et al.,* 2005, Gross, 2014, Fotopoulos and Pavlidis, 2015, Muñoz *et al.,* 2003, 2010).The distribution of viruses causing tumors of the genital organs (cervix, vagina, mouth cancer) is the following: HPV-16 occur in 54 %, HPV 18 in 17.2%, HPV 45 in 6.7%,HPV 31 in 2.9%, HPV 33 in 2.6% (Smith *et al.,* 2007). The presence of the virus can be detected in new born babies of infected mothers, as the virus can get from the mucus of birth channel into the oral cavity where viruses can cause laryngeal or pharyngeal tumor.

Less aggressive types (HPV 6, 11, 42, 43 and 44) can cause warts (Fig. 2) or benign tumors, however large and very disturbing skin outgrowth in the oral cavity or around genital organs (Fig. 3).

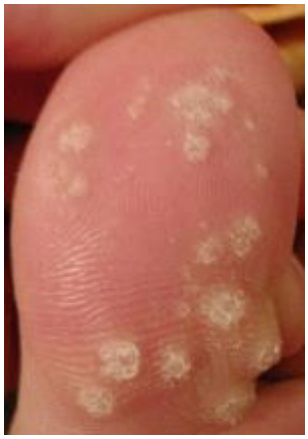

Figure 2. HPV infection of the skin, warts can be observed on the skin surface *(http://en.wikipedia.org/wiki/Wart).*

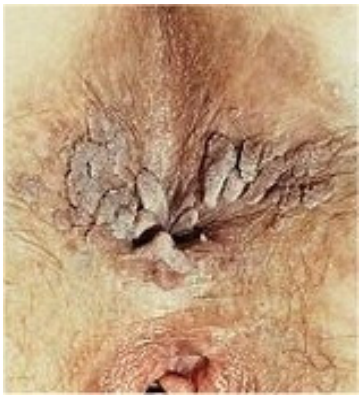

Figure 3. Anal warts *(http://en.wikipedia.org/wiki/Genital_wart)*

HPV detection methods

The anogenital virus infection can be detected by Papanicolau test. Characteristic cells can be seen in vaginal smear in case of infections (Fig.4). The core of infected cells is higher and a homogeneous light edge can be observed around of the core. It is surrounded by deep cell plasma. Infected cells generally have several cores. These cells are called koilocytes. The evaluation is performed with an optical microscope and it requires a lot of practice. Pink cells can be seen with two cell cores in the middle on the left of the photo below (Fig. 4).

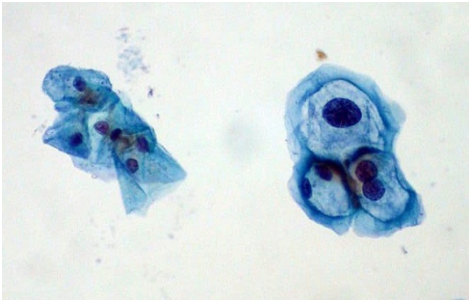

Figure 4. Papanicolau smear with group of normal cervical cells on left and HPV-infected cells showing features typical of koilocytes: enlarged nuclei and hyperchromasia *(http://commons.wikimedia.org/wiki/File:ThinPrep_Pap_smear_HPV.jpeg)*

If the Papanicolau test is positive, a specialist may suggest further tests like HPV DNA test. The HPV DNA test is very accurate because does not only show one strain, but the most common strains, if a multiplicity of infection has occurred (Wright and Schiffman, 2003). In the test a DNA extract is prepared in smears for the detection of various strains of HPV test strip.

This nucleic acid hybridization method utilizes a DNA cocktail specific for intermediate/high risk serotypes (types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Results are reported as not detected or as detected for high-risk HPV serotype. A not detected result is consistent with the absence of high-risk HPV DNA serotypes, a level of HPV DNA below the detection limit of the assay, or presence of a serotype other those listed above. A detected result indicates the presence of a one or more of these high-risk HPV serotypes (Saslow *et al.,* 2012).

Viral infection and replication

Most HPV viruses that infect a part of the body surface are covered with multilayer squamous mucous membrane (e.g. cervix, vagina, vulva, mouth, pharynx, larynx, esophagus, urethra and anus area). Papillomaviruses replicate and assemble

exclusively in the nucleus. Virus infects thekeratinocytes in the basal layers of a stratified squamous epithelium.

The viral DNA which has entered in the epidermis integrates into the germ layers dividing cells DNA and its replication starts in parallel tumble regulating epithelial cell proliferation operations. Towards the surface of the epidermis in the keratocytes the synthesis of viral components and assembly starts, and on the surface of the cell disruption newer viruses appear, and these new infected harness and an increasing number of virus are produced (Fig. 5).

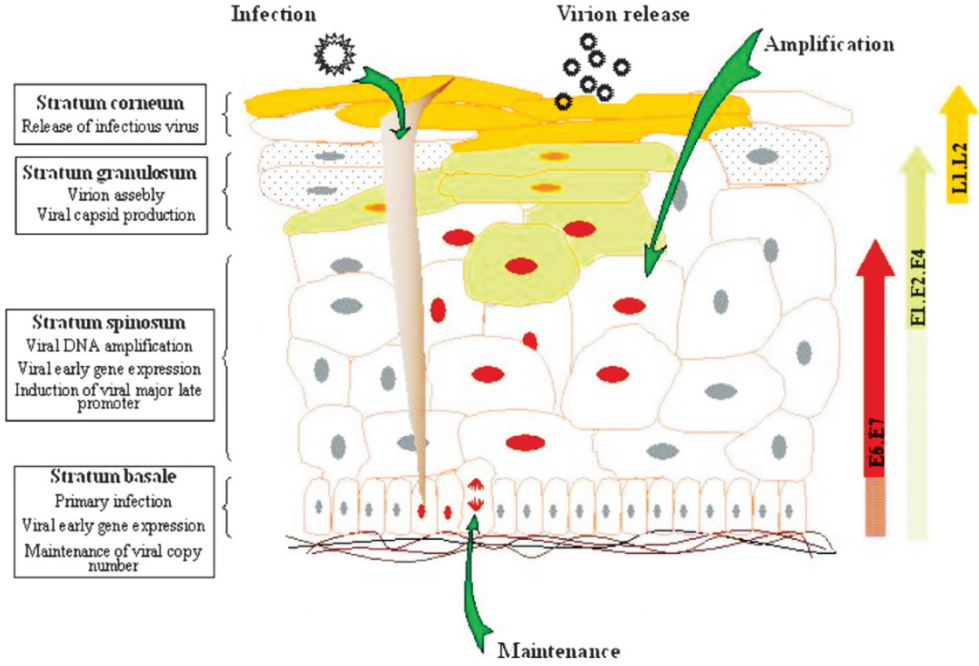

Figure 5. Stages of viral infection. The germ layer-dividing cells are already in the virus which is shared with the host cell. While wandering cells migrating to the surface of the epithelia the virus generates their constituents. The surface epithelial cells which have reached new viruses are released (Grm *et al.,* 2009).

It has been shown that the virus strains which cause genital tumors of E6-E7 protein coding genes are responsible for the oncogenic activity (Ganguly and Parihar, 2009, Hebner and Laimins, 2006). HPV once incorporated into DNA, inhibits the expression of regulators of E2 gene transcription, resulting in increased activity of E6-E7 genes (Grm *et al.,* 2009, Prétet *et al.,* 2007). E6, and E7 are viral oncogenes and their expression induces cell immortalization and transformation.

The proteins derived from transcription of genes, linked to the tumor suppressor proteins (p53 and pRb) in cell cycle stop regulation (Zheng and Baker, 2006). Following infection, cell division becomes out of control. The immune cells are not able to recognize the tumor cells. More and more abnormal cells are formed, which eventually lead to the appearance of malignant tumors.

Viral infection do not last lifetime, it can take the body several months or years to get rid of the pathogen if the person does not become infected again. If the body cannot get rid of the infection, triggered by the above-mentioned mechanisms it can lead to cancer induction process, which is slow, and can last decades.

How to defend ourselves against HPV?

In sexual intercourse it does not spread with body fluids, but with skin contact, so condom does not protect against the infection. For those who are sexually active (rather women than men) regular check-ups can be a lifesaver. Infected women partners can be at risk, on the one hand they can cause cancer in males, on the other hand there is a risk of re-infection in both sexes. Frequent change of partners increases the potential for the spread of the highly infectious (high-risk) viruses, and that at the same time even more virus strains are absorbed into the body.

Conclusions

Since mortality caused by HPV is higher than that of AIDS, the HPV threat to society should be informed and HPV should be handled more seriously. Globally cervical cancer is the second most common female cancer after breast cancer. HPV is the main reason of cervical cancer.

The majority of men infected with HPV can have symptoms and no symptoms at all, but certain types can cause genital warts and cancer. Public attention and that of students should be directed at the risk and treatment of HPV, as well as at the importance of prevention and screenings.

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