CLINICAL IMPACT AND IMPLICATION OF HUMAN PAPILLOMAVIRUS (HPV) IN CERVICAL CANCER

LILIANA PUSTAN[1], SIMONA DUNCA[2], OCTAVIŢA AILIESEI[2]

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Abstract: The social and economic evolution of the world's population in the last years has brought changes also in the prevalence of some diseases. Lately, viral infections have attracted specialists' interest due to the unexpected complications they cause. Until not so long ago incriminated only for the development of warts, the Human papillomavirus infections have been found also to induce cellular abnormalities, such as the koilocytes, which in their turn indicate low grade squamous intraepithelial lesions (HSIL). According to the latest assessments worldwide, the HPV is responsible for 70% of the cervical cancer cases. The extensive research studies conducted by specialists came to know success when the HPV vaccines were launched on the market. In the summer of 2006, the first vaccine able to stop the expansion of HPV-induced cervical cancer came out, GARDASILTM, produced by Merck and Co., Whitehouse Station, New Jersey. It is a tetravalent vaccine (generates immunity against the oncogenic viral types 16 and 18, and the non-oncogenic types 6 and 11). In our drugstores, one can find SILGARD, efficient and safe, providing 5-year protection, but not eradicating the effects of the viral infections acquired prior to vaccination. Apparition of the vaccine does not exclude cytological screening, which remains the most effective way to detect early a potential cancer of the cervix.

INTRODUCTION

According to the studies performed by the Clinical Epidemiology Unit RECIF of the "Gr.T.Popa" University of Medicine and Pharmacy Iasi, in Romania, the cervical cancer induces a rate of mortality 2 to 3 times higher than in the other EU member states. From this perspective, we have been ranked the first in Europe, and the second in terms of incidence. Each year, 2000 people die from this disease and 3000 new cases are detected and tested positive for malignancy. This is the also result of the poor organization of cancer screening at national, and particularly local level.

Starting with 1980, specialists have concentrated their attention on the involvement of the Human papillomavirus in cervical cancer cases. As a result, two HPV vaccines have been created: a bivalent one (against types 16 and 18), and a tetravalent one (against types 6, 11, 16, and 18). Despite being tetravalent, the latter provides immunity only to two oncogenic types of virus, namely 16 and 18 (actually the most frequent in Europe and USA), types 6 and 11 being responsible for anogenital vegetations. The tetravalent vaccine marketed also in our drugstores is SILGARD. It addresses young people, boys and girls aged 9 to 15 years old and young women aged 16 to 26 years old, and is administered preferably before they begin to engage in sexual activity. The researches have shown that the earlier in life the vaccine is administered, the higher the concentration of antibodies the body has, and therefore revaccination may be unnecessary any longer (Castle, 2002). The vaccination scheme includes a three-stage vaccination over 6 months, as follows: initial vaccine, 2 and respectively 6 months after the initial one. The research team from Merk Sharp & Dohme (MSD) who developed the vaccine are currently conducting clinical studies to cover also types 31 and 45 responsible for 9% of the cervical carcinoma cases. The vaccine does not create immunity to the types of viruses acquired before vaccination. They do refer, however, to a curative vaccination as well.

Human papillomavirus

Approximately 100 HPV types have been identified so far, some exhibiting a high risk of oncogenicity (about 20 types), others a lower one (see *Photo 1*).

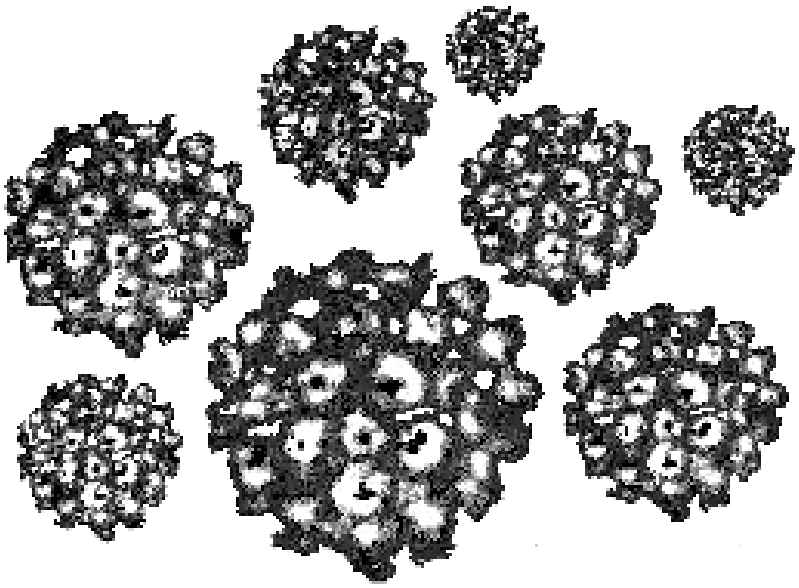

Photo 1. Electronic photomicrography of HPV

Types 16 and 18 are known as oncogenic, with high risk of producing cervical carcinoma (HR-HPVs), and most widely spread in Europe and USA.

Such viruses are small (50 – 55 cm), with double-stranded DNA and icosahedral capsid protein: L1 is the major protein, while L2 is the minor component.

Specialists focused on protein L1 which is capable of forming virus-like particles (VLPs) imitating the viral structure, but lacking virulence. They determine the immune response of the body, phenomenon based on which the HPV vaccine was created; the DNA encodes a number of 8 proteins, from E1 to E8, involved in virus replication. Replication takes place inside the cells of the intermediate and superficial layers of the mucosa, where they generate koilocytosis.

Generally, the virus shows the common characteristics of all viruses, namely they exhibit obligate intracellular parasitism, cross bacteriological filters, do not exhibit DNA and RNA simultaneously, cannot generate their own ATP, do not have ribosomes and are sensitive exclusively to interferon (do not respond to antibiotic therapy) (O' Brien, 2003). There are theories supporting the idea that the perinuclear halos specific to koilocytes develops as a result of the consumption of the cellular lactic acid by the energetic parasitic virus.

Epidemiology

Papillomaviruses are widely spread and are highly infective due to the fact they depend on the host cell both for ATP formation and RNA synthesis. Viral RNA replication takes place within the nucleus of the host cell by using the enzymes and cellular RNA-polymerase. Contamination may occur also by casual skin to skin genital contact. The host cell selected by the virus will be a basal cell from the germinative layer of the epidermis, the squamo-columnar junction. Viral DNA prefers an ever-changing area with cells which migrate to the superficial layers and maturate. If the selected cell is “defective” and does not maturate, the infection is compromised and spontaneous healing occurs. On the other hand, viral DNA may persist in the infected cell without producing visible lesions. If the evolution is normal, the virus comes to infect also the intermediate and superficial cells, forms koilocytes and thus can be detected by a cytologist. Target cells have two prospects: if they allow replication of the viral DNA, they do not suffer malignant transformations, and are subsequently eradicated; if they do not allow the replication, they undergo such transformations. The malignant tissue contains only cells with DNA integrated into the E1 and E2 proteins. Oncogenic HPV types stimulate cytokine production, which inhibit the local immune response, thus preventing the apparition of specific antigens. That is why the body’s defense capacity reduces, infection becomes persistent and pre-cancerous changes can appear. Since post-infective

immunity does not last, re-infection is extremely frequent. The vaccine addresses precisely such situations by alerting B and T-helper lymphocytes and providing the body with the necessary immunologic memory (Dunca, 2005; Pagliusi, 2004).

MATERIALS AND METHOD

HPV infection detection methods

The first and most effective method to detect infections caused by this virus is the one provided by cervico-vaginal cytology, which, in addition to the advantage of detecting the infection induced by the virus, it enables early identification of any type of cervical cancer. It is advisable that virus detection be conducted by cross testing which includes a cytological test, colposcopy, and DNA hybridization assay. Detection may be carried out also using either the Hybrid Capture (HC) test or the Polymerase Chain Reaction (PCR). The former consists in the identification of a light signal of intensity directly proportional to the viral content. A HC II kit has been launched to the market; it includes a sampling brush, a transport medium, and the solution of the hybridization technique based on the sequences of RNA synthesized in the cells corresponding to the sequences of viral DNA. PCR is based on the titration of the cellular polymerase involved in the replication of viral DNA.

All these techniques have equally advantages and drawbacks, thus underlying once again the importance of prophylactic vaccination both of girls and boys before starting their sexual life.

HPV infection can be detected by means of the Babes-Papanicolaou test, colposcopy examination or serum testing. Cytological examination is able to identify the presence of koilocytes representative for the infection with the virus. Colposcopy can detect a colpitis, but cannot provide any indication in terms of the etiologic diagnosis. Serum tests are able to detect the presence of the virus, but they should be followed by other tests designed to establish the type of the virus and classify it as oncogenic or not.

The samples are collected in gynecology clinics. It is crucial that samples be collected from the entire squamo-columnar junction area, since the virus is most commonly detected in this transformation zone. Moreover, it is in this zone that neoplasia develops most frequently. Two or four slides are sampled, depending on the gynecologist’s indications. In conventional cytology, it is recommended to affix the slides using a fixative spray or ethyl alcohol and leave them to dry in open air for a few minutes before transferring them to the cytology laboratory where they are stained using the Papanicolaou technique.

Papanicolaou’s staining

Wash with alcohol 80^0 _____ 30 sec.
alcohol 70^0 _____ 30 sec.
alcohol 50^0 _____ 30 sec.
Rinse with distilled water _____ 30 sec.
Stain in Harris’ hematoxylin _____ 3 – 6 min.
Wash with distilled water _____ 30 sec.
Submerge in 0.25% solution of hydrochloric acid _____ 6 times
Rinse with tap running water _____ 6 min.
Wash with distilled water _____ 30 sec.
Wash with 50% alcohol _____ 30 sec.
Wash with 70% alcohol _____ 30 sec.
Wash with 80% alcohol _____ 30 sec.
Wash with 95% alcohol _____ 30 sec.
Stain in Orange G _____ 90 sec.
Wash with 95% alcohol _____ 30 sec.
Stain in EA 50 _____ 90 sec.
Dehydrate in 95% alcohol _____ 30 sec.
Dehydrate in absolute alcohol _____ 30 sec.
Dehydrate and clear in solution of xylol and alcohol (1:1) 30 sec
Clear in xylol _____ 30 sec.
Mount in Canada balsam

This is the traditional Papanicolaou staining method, but some kit manufacturers have brought modifications specific to the products they manufacture.

Following the staining process, the cell nuclei are grayish blue to purple, while the cytoplasm is greenish blue in the cyanophil cells or orange-pink in the eosinophil ones.

RESULTS AND DISCUSSIONS

The prepared smears are examined under an optical microscope using 7 x oculars, the 10 x objective lens for the general picture, and 20 x for usual The cytologist will examine and evaluate the cell component, specifying the type of cells found on the slide and their state current of normality, as well as the inflammatory component and the microbial one. The presence of koilocytes is investigated in particular. These are HPV-infected, intermediate and superficial squamous cells with specific features: hypertrophic to gigantic nucleus with no apparent nucleoli, towards the periphery of the cell. In some cases, it can exhibit binucleation, but not multinucleation. The central and most of the area within the cell is occupied by a perinuclear halo, a void optical zone created by the parasite, clearly demarcated at the periphery by concentrated cytoplasm giving the impression of a thickened cellular wall (see *Photo 2*).

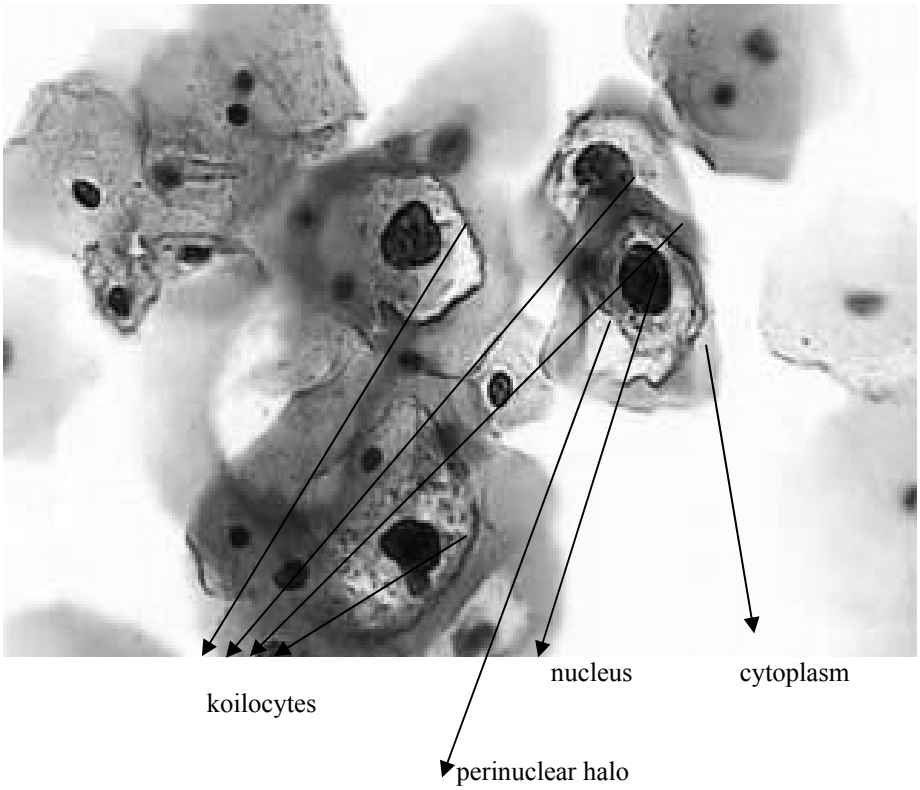

Photo 2. Vaginal smear prepared using Papanicolaou staining

The result of the microscopic examination will comprise classification according to the Bethesda system. This system was recognized by the International Academy of Cytology to the purpose of having a uniform and standard terminology for reporting cervical or vaginal cytologic diagnoses. To come to the support of specialists, a website called CYTOPATHNET was

developed. The site includes all the updates to the classification system in cervico-vaginal cytology.

Cytology in HSIL

Low grade squamous intraepithelial lesions (LSIL) occur most frequently in cervico-vaginal cytology. After the treatment, approximately 70% of them are cleared, but if neglected, they may develop into high grade squamous intraepithelial lesions (HSIL) (Teleman, 2007). The smears belonging to HSIL are characterized by the presence, and not the frequency, of intermediate and superficial squamous, dyskaryotic, hypertrophic cells with slightly cyanophil or eosinophil cytoplasm, large nucleus, but with maintained nucleus-cytoplasm ratio. Multinucleation may occur, but the nuclear membrane remains smooth, with finely granular, slightly hyperchromatic chromatin. Keratinization can also appear, manifested by dense, orange cytoplasm with picnotic nuclei. The inflammatory component can be well represented by neutrophil granulocytes, possibly by lymphocytes, or moderate (Reda, 2004). Detection of koilocytes on smears implies the inclusion of such smears in the HSIL category of the Bethesda system.

CONCLUSIONS

HPV induces a wide variety of lesions, benignant or malignant, from epithelial verrucas to invasive carcinoma.

Serological studies have detected the presence of the viral DNA in cases found negative at cytological and colposcopic examinations. That is why it is important to associate the Papanicolaou testing to viral type identification.

The vaccine is an efficient way to prevent infection with this virus, but it does not provide protection against other virus types except those for which it was developed, does not create lifetime immunity, nor acts against infections already existing before vaccination.

All the findings prove once again the importance of the annual screening and cervico-vaginal smears in cytological testing with a view to detecting, as early as possible, any pre-malignant or malignant lesion.

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1 – Regional Emergency Hospital, Pathological Anatomy Laboratory

2 – “Alexandru Ioan Cuza” University Iaşi, Faculty of Biology, Microbiology Laboratory