Human papillomavirus the challenge of the XXI. century

Ph.D Theses

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1. Introduction

Cervical Cancer
The human papillomavirus (HPV) infection is one of the most frequent virus related, sexually transmitted disease responsible for the development of more than 6% of all cancerous, mostly cervical cancer illnesses annually. The issue of cervical cancer has been raised again recently, as opposed to other states of the European Union our country shows a gradually increasing tendency in the mortality rate of cancerous diseases, even in respect of those, which could be screened. In the 21st century not a single woman should die of cervical cancer.

Cervical cancer is the collective term for malignant tumors developing on the portion, which claims the lives of approximately 500 women in Hungary annually until this day. The most typical point of development is where the columnar epithelium of the cervical canal and the squamous epithelium of the portion meet, the so called transformation zone (squamo-columnar junction).

The disease is a several year long process of squamous epithelium metaplasia. This is what provides the opportunity for screening, as by recognizing the lesion in a pre-cancerous state, treatment is possible prior to the development of a tumor. Most often we find squamous cell cancer, but we must not leave endocervical adenocarcinoma without notice either. The staging system of female genital organ cancers has been modified by IFGO several times. (1).

As it is known today, the development of a malignant tumor is a mutation of multiple points in the genome, which influences the process of cell cycle regulation, and which causes or inhibits apoptosis. A primary cause of cervical cancer is a virus, which entering the DNA modifies the process of cell division.

The Human Papillomavirus (HPV)
More than 100 strains of the human papillomavirus are know today. These are comprised of two primary parts. One is the capsule (capsid), which consists of 72 capsomers, and these capsomers are made up of two proteins (L1 and L2).

The second structural unit is the virus genome, which is a double-threaded ring shape made up of approximately eight thousand nucleotide pairs. The papillomavirus does not have serotypes, immune selection develops through years, and it is the result of cytotoxic immunity.
The regulatory section of the DNA, the non coding region (LCR) is responsible for its various degrees of causing tumors. From among the genes coding the early proteins, E5, E6 and E7 are the oncogenes of HPV. The oncoproteins coded by these bind the p53 tumor suppressor genes of the host-cell, thus causing unregulated cell proliferation. The HPV virus only infects the epithelium, and primarily in the fast dividing cells.

Breakage of the virus genome and damaging of the E2 gene causes the virus to merge into the cell DNA (covalent integration) and produce the conditions for malignant degeneration. Chromosome 8 and the c-myc oncogene also participate in disordered cell growth and the process of development of cervical cancer.(2)

From among the previously mentioned 100 strains 30% may be accounted for genital infections. Genital HPV is only transmitted through sexual contact. The strains of the virus are divided into low-risk and high-risk groups, which are differentiated on the basis of probability of development of a cervical disease. Low-risk factor types (6, 11, 42, 43 and 44) cause benign condiloma.

Types with a high-risk factor (16, 18, 31, 33, 35, 39, 45, 51, 56, 58, 59 and 68) are found in 99.7% of cervical cancer tissues.(3)

Based on statistical results in Hungary more than 5000 new HPV infection cases are found annually. (Table 1.)

<table>
<thead>
<tr>
<th>Year</th>
<th>Female</th>
<th>Male</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
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<td>2776</td>
<td>674</td>
<td>3450</td>
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<tr>
<td>2005</td>
<td>3107</td>
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<tr>
<td>Total:</td>
<td>18330</td>
<td>4639</td>
<td>22969</td>
</tr>
</tbody>
</table>

Table 1.
2. Objectives

The focus of the thesis is to study the prevalence of the human papillomavirus (HPV) and the methods of disease diagnostics, determination of the HPV type in the group of reproductive aged, cytologically negative, healthy females.

We have also investigated the risk factors of cervical cancer in relation to the prevalence of the human papillomavirus.

Detailed methodology and results are introduced under the respective headings, based on publications in the given subject. Based on our results we shall position the method within the process of gynecological cancer screening.
3. The diagnostic and prevalence of genital human papillomavirus (HPV) infection in Hungary

Abstract

Objective: To show the prevalence and determine the type of human papillomavirus (HPV) in healthy women of reproductive age in Hungary. Study Design: We determined HPV nucleic acid using the Digene Hybrid Capture HPV-DNA assay from endocervical swabs of 1120 volunteer women of reproductive age. With the help of the hybridization antibody capture test we determined 14 HPV types (low risk, intermediate and high risk). Results: HPV prevalence was 17.5% considering the whole material. At the Szeged center 27.6% of the women screened were HPV positive, whereas at the three centers in Budapest, HPV prevalence did not exceed 15% in either of them. With a cytological examination out of 1100 cases, 117 (10.6%) were found to be HPV infected. The virus infection could be shown out of 1018 non-malignant cytologies in 60 (5.9%) cases and from 82 epithelial lesions 57 (69.5%) were infected. The cytological and molecular HPV diagnoses showed a significant relation to each other (P < 0.001). The cytological method showed HPV infections with a low degree of efficiency (sensitivity: 23.8%). On the other hand, the specificity (92.2%) is an acceptable method for the real negativity of the light microscopic HPV infection. Conclusions: These facts mean regarding the detection of HPV-DNA genoms that HPV positive cytological reports are false negative and in dysplasias are false positive. Since in HPV infected women the development of CIN is a great risk, it is advisable to carry out the HPV determination and typing in the so-called „endangered” groups.
4. Detection of human papillomavirus infection by the nucleic acid hybridization method (a multicenter study)

Abstract

Detection of human papillomaviruses with hybrid capture assay (a multicentre study). The human papillomaviruses (HPV) are regarded as one of the the important agents of cervical carcinoma. A multicentre study was organized to determine the prevalence of HPV in the fertile female population in Hungary. Parallel with the clinical sample collection, a questionnaire interview was performed to acquire data on the life style, socioeconomic status, sexual practice, etc. 1200 women were examined colposcopically and cervix samples were collected for cytology and the detection of HPV DNA. 17.4% of the samples were HPV-infected. 3.9% of the patients had acquired low-risk, and 10.1% 10.2% high-risk HPV types; 3.4% of the women were at the same time infected with both low-risk and high-risk HPV types. Simultaneously performance of cytology and the HPV hybrid capture assay contribute to recognise and treat the precancerous status and risk factors.
5. Human papillomavirus (HPV) the challenge of the XXI. century

Cervical cancer is the second most frequent type of tumor among women world-wide, and even these days it is one of the leading causes of death. HPV has a major etiological role in cervical carcinogenesis. About 100 types of HPV are characterized. Types responsible for genito-mucosal infection can be clustered into three categories. Low-risk HPV types cause benign alterations. There are types in the medium-risk group. In the high-risk group there are four HPV types (HPV-16, -18, -45, -31), which are responsible for 80% of cervical tumors world-wide. Prevalence of HPV is highest in the age group of between 20-25, and it slowly decreases with advancement of age. There is at least an 80% chance, that woman go through a HPV infection once in their lives. Significant portion of these diseases however, is only subclinical. After an HPV infection long time passes until development of a tumor. Regular gynecological screening is highly important in order to notice the disease during the cancer prevention stadium. The traditional methods of gynecological cancer screenings (inspection) are taking of the a swab (cytology), and coploscopy. In view of results further examinations and therapy can be decided on the basis of screening principles in accordance with the guidelines of the Obstetrics and Gynecology Specialization Academy.

<table>
<thead>
<tr>
<th>Clinical forms of the genital human papillomavirus (HPV)</th>
</tr>
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<tbody>
<tr>
<td>Clinical form</td>
</tr>
<tr>
<td>Clinical</td>
</tr>
<tr>
<td>Subclinical</td>
</tr>
<tr>
<td>Latent</td>
</tr>
</tbody>
</table>

Table 1.

The Professional Academy of Obstetrics and Gynecology has accepted principles for cancer screening.

Bőszé, P; Gőcze, P; Hernádi, Z; Pap, K; Ungár, L.: Aspects of Cervical Cancer Screening: national principles. Guidelines of the Professional Academy of Obstetrics and Gynecology. (A méhnyakrák szűrésének szempontjai:
hazai irányelvek. A Szülészeti és Nőgyógyászati Szakmai Kollégium útmutatója.) Gynecological Oncology (Nőgyógyászati Onkológia 2009; 14:11-17).
6. Summary

Possible Future Approach
Solutions are sought in Europe, which may improve and facilitate screening, making it more sensitive and specific. Traditional cytology is mostly subjective, and it significantly depends on the person and experience of the one performing the examination. There have been several trials so far to replace the method with one baring higher sensitivity and specificity.
One such trial is fluorescent spectroscopy, which is based on the auto-fluorescence of NADH, FADH, elastine and collagen, as well as absorbent hemoglobin, on the basis of which their quantities may be determined through measurement. Since the quantities of these vary in sound pre-tumor state and tumorous state, the two can be differentiated.
The advantage of the process is that it is non-invasive, and it gives immediate results, as the extension of the deformation is visible in vivo, making it viewable by pointing monochromatic light onto the cervix through a fiber-optic cable. Sensitivity of the method is 86% with a specificity of 74%, thus it is more sensitive than average cytological methods.
Raman type spectroscopy is based on a similar theory, predestined to improve specificity. Inflammation or benign lesions may give fake positive results with the previous method, and this is what the latter prevents.
The methodology is based on the Raman type, or inflexible dispersion, originating from the vibration or rotation related transformation of molecules. Spectroscopy evaluates the energy differences between the original photon and the one entering the tissue, and gives results in cm$^{-1}$ units. The advantage of the method is that all molecules present in nature are Raman-active molecules with a typical spectrum in the close infrared range. In vivo Raman spectrum may be recorded in 90 seconds. Further areas of research in this respect are the examination of tumors of the colon, breasts, brain, vesicle, uterus and ovary. In spite of this fact the cheapest and most frequent method of screening in Europe is the Pap-smear test.(1)

Status of Screening in Europe
In states with well organized screening (Denmark, Finland, Iceland and Sweden) the occurrence of cervical cancer has evidently decreased as opposed to Norway, where screening is in an immature stage. In the United Kingdom, where the rate of women participating on screening examinations has increased from 25% to 85% since 1980, an annual 7% decrease has been achieved in the rate of mortality, this tendency continuing until this day. (2)
Unfortunately our country is among the weakly screened nations, not even close to the 80% screening rate recommended by WHO for decreasing mortality in Europe. In the initial years of the cervix program, screening has reached a 50% proportion, however this does not seem to show any increase, rather a decreasing tendency is prevalent.

Groups of the National Health Administration (ÁNTSZ) involved in screenings are seeking ways to increase the number of women participating on examinations. Several methods have been developed for such aims, ones such being the improvement of communication and stressing the importance of screening examinations on all forums. In educational institutions such awareness could be part of health-care instruction, and knowledge could be made more wide-spread through media. Making the new screening program continuous in the short-run, active participation of the population on HPV-screenings would be essential. Human and material conditions are given, people must only be made aware, that in case of an early and accurate diagnosis, the appropriate therapy could bare positive effects both on possible disease processes and prognosis. Assertion of the role of vaccination and providing the HPV vaccine to the young endangered groups is advised. Specific screening guidelines support the work of cancer screening professionals as well, nevertheless this may only succeed if women do attend the gynecological screenings in order to prevent HPV related illnesses.

There may be numerous reasons for the ineffectiveness of screening, one being disinterest on behalf of women, which could be notably improved with adequate consultancy. Health-care workers may participate in this as well. Significance of screening has to be proven to the population, thus improving mortality statistics, as it is unacceptable, that women still die of an early revealed and 100% curable disease in the 21st century.
7. New Results, Benefits in Practice

Our research presented in the thesis provide new results and opportunities for new practical usage.

Results received in the course of our work may be summarized as follows:
I. With consideration to the entire material HPV prevalence was 17.54%. Risk factors regarding HPV infection were the large number of sexual partners, age, education, and the time of starting sexual activity. Since there is a greater chance of CIN development in HPV infected women, finding and determination of the type of HPV is recommended in the so called "endangered" groups. Molecular virology results carry benefits both in prognosis and therapeutic consequences.

II. Considering the fact, that 17.4% of examined women were found to be infected with the human papillomavirus (3.9% low, 10.1% high, 3.4% low and high virus types acquired), the HPV nucleic acid hybridization test carried out along with cervix cytology helps recognition and treatment of pre-cancer states and risk factors of the cervix.

III. It is advised to start attending gynecological cancer screening two years after starting sexual activity, but from 21 years of age as the latest. The smear test is recommended every two years in the conceptive age and every 3 years after menopause.

Evaluation of a positive report and tasks according to national guidelines (Professional Academy of Obstetrics and Gynecology).