Cervical cancer

Cervical cancer is the second common cancer among women worldwide, with almost half a million new cases each year. Almost 80% of the women affected are in the developing world. However, many of these cases could be prevented from progressing to invasive disease and potential death. Among the east European countries with the increase of cervical cancer incidence Serbia is on the top of the list. Serbian data from year 2002 shows the standard incidence rate of 27/100 000 women, and in some regions even 41/100 000 women (Braničevo region). According to Vojvodina Register for Malignant Neoplasia we discover 280 new cases of cervical cancer in our region every year and 145 women die due to the consequences of the disease. The top place of our country on the list of all countries in Europe is related to the consequence of a longstanding increase of number of women with cervical cancer and ineffective measures that was carried out. More than any other cancer, cervical cancer is a disease which lends itself to early detection and treatment. The effectiveness of cytology screening as a method to reduce the number of invasive cases and deaths resulting from cervical cancer in developed countries has already been demonstrated (1).

HPV infection

HPV is the most important known predisposing factor for the appearance of cancer of: cervix, vulva, anus, penis, and extragenital organs such as mouth and esophagus (2-9). The presence of some types of HPV in woman’s genital system is connected with numerous diseases condyloma, Bowenoid papulosis, cervical, vaginal, and vulvar intraepithelial carcinoma. There is general acceptance that all types of sexual intercourse can be the main way in transmission of most HPV types. Historically, HPV 16 and 18 have been regarded as high risk cancer associated HPV types. HPV types 31, 33 and 35 have been demonstrated to have an intermediate association with cancer. This intermediate association is due to the fact that these types are more frequently detected in cervical intraepithelial neoplasia CIN2 and CIN3 rather than in cancers. These five HPV types together account for about 80% of cervical cancers.

Additional high- and immediate-risk HPV DNA types, including types 39, 45, 52, 56, 58 and 68, have been identified as the principal HPV’s detectable in other cancers.

HPV testing in cervical cancer screening

Within the past 5 years, guidelines recognizing the value of HPV testing in both primary cervical screening and in the management of abnormal cervical cytology have been established in the US and are being considered in Europe (10-14). This trend has occurred because of the definitive association of high-risk human papillomavirus (HR HPV) with cervical cancer and the evidence that the sensitivity of HR HPV testing for neoplasia grade 2 or more severe (CIN 2+) is substantially higher than cytology testing (15,16). Higher sensitivity offers a number of advantages, including, most importantly, the potential of reducing cervical cancer rates while reducing the number of screens in a lifetime necessary to achieve this goal.

Underdeveloped and developing countries, which have the highest incidence of cervical cancer, traditionally uses Pap test and cervical visualization method using vinegar and iodine, as the most economical tests in cervical cancer screening (17). In our country, decision of using hc2 DNA test in a pilot project is recommended and is based on studies that confirm significant decrease in number of recurrent tests in woman with border Pap findings. It leads to less frequent need for Pap testing, even every 3-5 years for woman with negative HPV test, which results with significantly less costs of all cervical cancer screening (18-21).

The possibilities of new technologies in diagnostics and high incidence of cervical cancer in Vojvodina were the main reason to start the pilot program DECENA at the Oncology Institute of Vojvodina in 2006. In prescreening...
phase of DECENA pilot program, we focused on how to increase efficacy of screening program.

The analysis of the possibilities for maintaining better efficiency in diagnostic of premalignant diseases shows limitations of cytology methods. In most European countries, there was noticed even more than 30% of false negative Pap test in the group of all diagnosed cervical cancer cases, and the additional 10% is due to mistakes in analyzing the atypical cytology findings (22,23). Despite of all these limitations, there is nevertheless the significant decrease in number of cervical cancer diagnosed woman, thanks to Pap test, still the “golden standard” in all traditional screening programs. After analyzing the costs of standard screening, a few national screening committees recommended:

– to decrease the frequency of screening in woman with regular cytology testing
– new cytology methods, like liquid-based cytology, for higher sensitivity
– new testing methods of HPV HR, as this virus is the main cause of cervical cancer

Principles of Hybrid Capture 2 DNA test

HPV testing along with a Pap test is emerging as the standard of prescreening and is acknowledged in clinical guidelines developed by major medical groups including the American College of Obstetricians and Gynecologists (ACOG), the American Cancer Society (ACS), the Association of Reproductive Health Professionals (ARHP), and the American Society for Colposcopy and Cervical Pathology (ASCCP) (24).

The Hybrid Capture 2 HPV DNA test using Hybrid Capture 2 technology is a nucleic acid hybridization assay with signal amplification that utilizes microplate chemiluminescent detection. Specimens containing the target DNA hybridize with a specific RNA probe cocktail. The resultant RNA:DNA hybrids are captured onto the surface of a microplate well coated with antibodies specific for 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, which 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.

Hybrid Capture technology is illustrated below (Figure 1).

DECENA program

Hc2 DNA test for determination of 13 oncogenic human papillomavirus genotypes was introduced in the Oncology Institute of Vojvodina in August 2006. Another four systems for hc2 DNA test were installed in Health Centers in Senta, Sombor, Sremiska Mitrovica, and Zrenjanin. Education for specialist of genetics, molecular biologists, and pathologists working on this topic was also provided.

During August 2006 to April 2007 we tested 980 women. They were included into the pilot study of the primary screening procedure. This is a first study of this kind in our country.

Criteria for inclusion in DECENA program were:

– women aged from 30 to 44 years
– women with no previous treatment of cervical cancer
– women with no previous hysterectomy
– pregnant women
– women who gave a birth 8 weeks before
– women in assisted reproduction procedures

The main objective of DECENA pilot study was to estimate whether HPV DNA test application together with Pap test will increase sensitivity of cervical cancer primary screening.

All included patients were provided with a detailed explanation of the procedure. After giving their written consent and completing the questionnaire, the patients were examined for Pap test, vaginal discharge, and colposcopy according to decision of the gynecologist. The obtaining the results of Pap and HPV tests the patients were treated according to institutional protocol.

High risk types of HPV are the main risk factors for development of cervical cancer. Even 10% of women with normally epithelialized cervix are HPV DNA positive, and real incidence of HPV positivity depends on age and some demographic specificity (25,26). Prospective research studies show that 15% to 28% of women with positive HPV DNA test develop squamous intraepithelial neoplasia (SIL), comparing to only 1% to 3% of women negative to HPV DNA. It
is noticed that women positive to HPV 16, 18 have much higher SIL risk, compa-
ring with those who are positive to other HPV types (21,27). These types of
studies are important because they are connected with a lot of questions
related to genotyping and application of preventive vaccines in everyday prac-
tice. Molecular-microbiological methods present the best choice in diagnostics
of HPV infection. The most reliable methods used for detection of various
genotypes are: In Situ Hybridization, Southern Transfer Hybridization, Hybrid
Capture, Dot Blot, Filter Hybridization, and Polymerase Chain Reaction (PCR).
HeC2 DNA test is the only method approved by FDA (United States Food and
Drug Administration).

It is necessary to emphasize the importance of good communication with
women. Each woman has to be well informed about high prevalence of
this infection, especially among those younger than 30 years; possibility of
spontaneous elimination of virus from the body; longstanding evolution of
premalignant and malignant lesions, and also that the presence of HPV infec-
tion does not mean persistence of cervical disease.

Preliminary results of our pilot study are very encouraging for continuation of
our work. Obtained information is the same as we predicted: 16% of women
are HR HPV positive, which is in correlation with the data from surrounding
countries. HR HPV status is important in group of nullipara with cervical
biopsy CIN 2 changes especially when making decision about their adequate
treatment. In this group there is even 89% of positive HR HPV test (values
ranged between 470 and 2020 RLU/CO).

We point out to women’s very good response to the screening test, and also to
the fact that they were well informed about cervical cancer and HPV. The most
of them were completely informed about this subject through media, and only
8% from medical professionals. It is necessary for additional activities, which
to have to be inserted in our future work plans. The most important problems
during realization of DECENA screening program were related to inadequate
communication between health workers and women. Medical professionals
should be trained how to adequately inform women about HPV infection and
cervical cancer genesis. Detailed questionnaire, adapted to socio-cultural
characteristics of our women, will surely enable epidemiologists, social work-
ers, and psychologists to give us important and useful information.

New screening study (TOMBOLA group) points out another problem - a
psychology problem in some women, due to the border Pap findings (28).
In spite of fast treatment procedure after the positive biopsy findings, two of
our patients with detected H-SIL changes, needed consultation with clinical
psychologist and therapy.

Until today, there is yet no absolutely certain and reliable way to distinguish women
with the high risk of developing cervical cancer. So, identifying that group of women
is important, because it makes better chances for prevention and treatment.

Information of HR HPV status is of great help for clinician who makes deci-
sions for observing or treating the patient, even though this fact also pretend
to be potentially upsetting information for woman. Described molecular-
diagnostic method requires laboratories with modern equipment, and well
educated medical professionals.

Conclusion

The results obtained from the use of DECENA program are:

1. With this program we made possible diagnosis of premalignant lesions in
women with accurate Pap test.

2. The program made possible to set up histopathological diagnosis in
shorter period than it was before, without repeating Pap test.

3. Giving of informative booklet about Pap test and oncogenic human papillo-
mavirus testing is adequate tool for instigating women to involve in screening
program and for hold on in procedure. Women informing is necessary tool in
primary screening of cervical cancer.

4. Women answers to questions from the questionnaire (their knowledge about
cervical cancer genesis, HPV vaccines) give us possibility to create further strat-
egy in informing of women and their partners about cervical cancer prevention.

Serious analysis of medical findings and epidemiologic data will surely give
us good direction for applying and using new technologies in cervical cancer
prevention. It is recommended to make testing only by indications that are
confirmed by clinical study, and also status of oncogenic human papilloma-
virus should be used only in context of other diagnostic procedures (cytol-
ogy, colposcopy). Application and using of new methods in cervical cancer
screening program should be well observed and controlled.

Conflict of interest

We declare no conflicts of interest.

REFERENCES

American College of Obstetricians ad Gynecologists; 2003.
2. Lerma E, Matas-Guix X, Lee SJ, Prat J. Squamous cell carcinoma of the vulva:
4. Palefsky JM, Holly EA, Rabston ML, Jay N. Prevalence and risk factors for human
papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-
of circumcision, medical conditions, and sexual activity and risk of penile cancer. J
et al. Association of human papillomavirus with penile carcinoma: a study using
papillomavirus and oral cancer: the International Agency for Research on Cancer
Consensus Conference. 2001 Consensus guidelines for the management of women
guidance for the use of human papillomavirus DNA testing as an adjunct to cervical
European and North American studies on HPV testing in primary cervical cancer
Management of women who test positive for high-risk types of human papillomavi-
14. von Knebel Doeberitz M. New markers for cervical dysplasia to visualize the genomic
chaos created by aberrant oncogenic papillomavirus infections. Eur J Cancer
2002;38:2229-42.
Randomized controlled trial of human papillomavirus testing versus Pap cytology in
the primary screening for cervical cancer precursors: design, methods and prelimi-
nary accrual results of the Canadian cervical screening trial (CCCaST). Int J
Cancer 2006;119:615-23.
auditing smear histories of women with and without cervical cancer. Br J Cancer