Review Article

HPV TYPING AS THE METHOD OF CHOICE IN THE PREVENTION AND EARLY DETECTION OF CERVICAL CANCER

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Abstract

Background: Human papillomavirus (HPV) is recognized as the primary cause of cervical cancer (CC), and significant coexisting factors have also been identified. CC is considered a curable disease because it has a long preinvasive period. It is possible to organize effective screening, and the treatment of preinvasive lesions is successful.

Aim: To show the importance of HPV typing in the early detection of premalignant cervical disease.

Method: Literature review, synthesis, and extraction of key professional and contemporary literature.

Results: Depending on the methods used in identification, the presence of different types of HPV was identified in about 90% of intraepithelial neoplasia and CC samples. HPV genotyping plays an important role in the detection of dysplasia and helps to reduce the number of false-positive Pap test results. Randomized controlled trials have shown that CC screening with HPV testing offers greater protection against cervical precancer and cancer compared with cytology-based screening. To date, it has been proven that HPV 16 and 18 genotypes have the highest oncogenic potential. These two genotypes are responsible for about 70% of all squamous cell carcinomas, 30–40% of vulvar cancers, and about 85% of cervical adenocarcinomas. According to a recent report, 48 (35%) of 139 countries recommended HPV-based cervical screening, with most currently switching from cytology to HPV testing.

Conclusion: HPV typing can be of great benefit in the early detection of malignant transformation of infected cells and the prevention of CC.

Keywords: cervical cancer, human papillomavirus, screening

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Introduction

Today, it has been proven that infection with oncogenic types of human papillomavirus (HPV) is the main etiological factor for the development of cervical cancer CC[1,2]. There are over 200 HPV genotypes, and only 50 infect the epithelial cells of the skin and mucous membrane of the anogenital region[3].

Papillomaviruses are DNA viruses and belong to the Papillomaviridae family[4,5] . The key event is the entry of the virus into the cell[6] . The virus enters the basal and parabasal layers of the epithelium through damage to the epithelium in the cervical transformation zone (Figure 1)[7]. The virion binds to the cellular receptor α6 integrin[8,9]. Different types of HPV penetrate cells by different mechanisms. The malignant potential of HPV depends on the frequency of viral integration and is different for different types of HPV. After the virus enters the cell, HPV infection can take three different forms: latent, which can only be detected by molecular biological methods (HPV typing), subclinical, which is diagnosed by colposcopy and exfoliative cytology, and clinical. Most genital infections are latent and subclinical[10,11].

Most HPV infections in young women resolve spontaneously, usually within a 24-month period. The heterogeneous outcome of epidemiological studies may be due to several important factors. First, there appear to be significant differences in HPV prevalence in different populations regarding age, frequency of cytologic abnormalities, and diversity of HPV genotypes. Second, multiple sampling and HPV-DNA detection techniques were used, with different sensitivities and specificities, which can significantly affect detection rates[12].

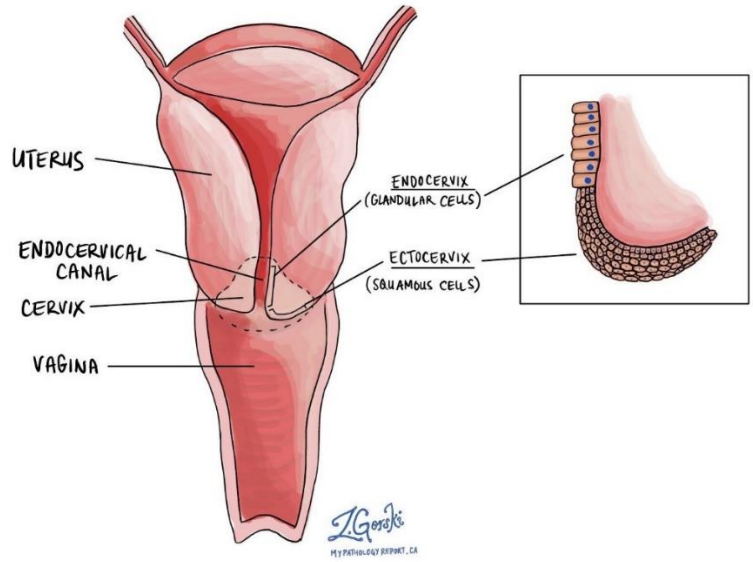
In over 99% of cases, the appearance of CC is preceded by persistent infection of the cervical epithelium with high-risk types of HPV[3,4]. In cases of persistent infection, there is a shedding of the viral ring and the integration of the viral genome into the host genome in the basal cells of the squamous epithelium[6,12,13]. The natural course of HPV infection, including the mode of transmission of the virus, the development of persistent infection, clearance of the virus, and interaction with the immune system, is only partially known. There is currently no established definition of persistent HPV infection. One study suggested that women with mild or moderate dyskaryosis should be referred for treatment only after persistent HPV infection for at least 6 months. However, the detection of HPV-DNA in a consecutive sample should include genotyping or even analysis of molecular variants to confirm the persistence of the same virus over time[13].

Molecular biological tests have proven that cervical lesions of low-grade LSIL (low-grade squamous intraepithelial lesion) differ from cervical lesions of high-grade HSIL (high-grade squamous intraepithelial lesion)[6,12]. In cases of HPV infection, 9 to 15 years pass from the appearance of initial, mild, and moderate premalignant low-grade cervical lesions of the LSIL type to the development of high-grade cervical lesions of the HSIL type and CC[14].

Based on epidemiological, molecular, and clinical observations on the association between HPV infection, premalignant cervical lesions, and CC[15], in 2003, the International Agency for Research on Cancer (IARC) proposed a division according to the oncogenic potential of HPV virus[16]. According to this division, types of low oncogenic risk include HPV types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and SR6108; types of high oncogenic risk include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82; and types 26, 53, and 66 are currently

considered to be of probably high oncogenic risk[15,16]. This categorization is not definitive[15] and changes with the discovery of new genotypes. Recent research indicates that the malignant potential of HPV depends on the frequency of virus integration and is different for different types of HPV. To date, it has been proven that HPV 16 and 18 genotypes have the highest oncogenic potential[17,18]. The oncogenic potential of HPV 16 is about 53%, and HPV 18 is about 18%. These two genotypes are responsible for about 70% of all squamous cell carcinomas, 30–40% of vulvar cancers, and about 85% of cervical adenocarcinomas[14]. Other HPV types have less oncogenic potential; HPV 31 is about 3%, and HPV 56 is less than 1%. The most common HPV types with low oncogenic potential are HPV 6 and 11 and they are detected in about 90% of genital warts (condylomas)[18]. These types are not associated with high-grade squamous lesions or CC.

Figure 1. Cervical transformation zone



The aim of this work was to show the importance of HPV typing in the early detection of premalignant cervical disease.

Method

Literature review, synthesis, and extraction of key professional and contemporary literature.

Results

Epidemiological aspects of CC risk

Sexual and reproductive characteristics. The risk factors for the occurrence of CC contrast with the risk factors for breast cancer: late menarche (after the age of 15), early first pregnancy (before the age of 18), a large number of births, and abortions[12]. The greatest association exists between the cervical canal, preinvasive lesions, and HPV infection. Depending on the methods used in identification, the presence of different types of HPV was identified in about 90% of intraepithelial neoplasia and CC samples[19].

Socio-economic status and ethnic origin. It is considered that socio-cultural habits have a significant influence on sexual habits as well as on reproductive characteristics. Unlike breast carcinoma, CC is more common in women with lower socioeconomic status[19].

Use of hormonal preparations. Several studies, including multinational studies by the World Health Organization, have given conflicting results on the impact of oral contraception as a risk factor[19]. It is believed that today, close to 61 million women in the world use oral contraception and that its long-term use is a risk factor for the occurrence of CC[20].

Nutrition. The most attention is paid to the amount of fat in the diet. Animal experiments have shown that high-fat foods increase the frequency and decrease the length of the latent period. In later case-control and cohort studies, a rather uneven relationship was found due to

confounding factors[19]. The results of epidemiological research indicate the protective action of fruits and vegetables[21].

Way of life and habits. Physical activity reduces the risk of CC[19]. Drinking alcohol 2-3 times a day increases the risk by about 25% compared to non-drinkers[20]. Many epidemiological studies indicate that smoking increases the risk, and the harmful effect of the number of carcinogens in tobacco smoke has been proven[19].

Family history. The risk of developing CC is 1.5-2 times higher in women whose first-degree relatives have the disease. The risk is twice as low if it is a second-degree relative. The risk increases if the disease is detected in a relative before the age of 40-45. years of life. The onset of the disease at a young age is the strongest indicator of genetic predisposition[19].

Importance of detection and genotyping of HPV

According to the data of the Cancer Registry in the Republic of Serbia for the year 2019, the incidence of the disease in Serbia is between 12/100,000 and even 35/100,000, the highest at the age of 35, but the highest age-specific disease rates are at the age of 50 to in the 59th year of life[20]. Detection and genotyping of HPV is important for detecting high-risk genotypes that cause CC. In Serbia, unfortunately, between 300 and 900 women die annually from CC[20] . Today, the detection of high-risk HPV is classified as a diagnostic method through three models[19].

1. The test of choice for women with a borderline cytological finding of the presence of atypical squamous cells of undetermined significance (ASCUS), atypical glandular cells (AGC), and squamous intraepithelial lesions (SIL) that will need further follow-up

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2. Primary test - alone or in combination with a Pap test for early detection of CC precursors.

3. Follow-up test after excisional treatment of H-SIL. It has been proven that a positive HPV test after the treatment of SIL has higher sensitivity compared to repeated cytology, and for this reason, the HPV test has been introduced into the follow-up protocols after the treatment of SIL, and double testing with a Pap test and an HPV test six months after the procedure is recommended. Growing knowledge about the relationship between HPV infection and CC has led to the creation of prophylactic vaccines aimed at primary prevention of the disease. Several commercial vaccines for different HPV types are in use today. There are known bivalent (against HPV 16 and 18), quadrivalent (against HPV 6, 11, 16, 18), and nine-valent (against HPV 6, 11, 16, 18, 31, 33, 45, 52 and 58) vaccines[12]. HPV genotyping plays an important role in the detection of dysplasia and helps to reduce the number of false-positive Pap test results. Standard laboratory diagnosis of HPV infection involves the application of tests to prove the presence of the virus, viral antigen, or viral nucleic acid in clinical material or serological proof of the presence of specific antibodies[19,20] (Table 1)

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Table 1. Diagnosis of HPV infection

Isolation: cannot be cultivated
Direct virus detection
Electron microscopy
Direct detection of viral antigen, PAP, IF, ELISA
Serological diagnostics - rarely used
Hybridization: Southern blot, dot blot, ISH, filter ISH
PCR

Screening

The goal of screening for CC is to reduce the number of cases and the number of deaths from this disease. In addition, early detection and successful treatment significantly improve the quality of life of women, enable future births, and, from an economic aspect, reduce treatment costs many times over. In countries where screening is well organized, the number of sick and dead women has been significantly reduced. The European recommendations are: starting screening between the ages of 20 and 30 and regular Pap smear examinations every 3 to 5 years, until the age of 60 to 65. The screening program should be organized, with quality control at all levels.

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In 2020, the World Health Organization (WHO) announced a global strategy to eliminate CC as a public health priority by reducing the incidence of CC to less than 4 per 100,000[21]. Over the past 40 years, secondary prevention by screening and treating preinvasive lesions has contributed to a significant reduction in incidence and mortality. Primary prevention with HPV vaccination is expected to further reduce the incidence[22]. In line with WHO recommendations, the Canadian Partnership Against Cancer has proposed an action plan to eliminate CC in Canada by 2030[23]. Priorities of this plan include the implementation of CC screening which primarily uses HPV testing, and improved follow-up on abnormal screening results. Primary HPV testing is not currently performed but is expected to happen soon.

The introduction of cytological screening programs reduced the incidence of CC. However, these programs have failed to eliminate CC due to problems including test limitations and suboptimal coverage of screening activities. Randomized controlled trials (RCTs) have shown that CC screening with HPV testing offers greater protection against cervical precancer and cancer compared with cytology-based screening[24-27]. In 2021, the International Agency for Research on Cancer (IARC) reported that, in general, HPV DNA testing is more sensitive than cytology for detecting H-SIL and is associated with reduced rates of detection of these high-risk changes in repeat screenings, as well as greater rates of reduction in cervical cancer incidence than cytological analysis when using the same screening interval[28]. According to a recent report, 48 (35%) of 139 countries recommended HPV-based cervical screening, with most currently switching from cytology to HPV testing[29].

Molecular testing for HR-HPV DNA can detect infection very early. Given that only a small number of women develop a disease that progresses to cancer, there is interest in defining secondary markers that have potential application in identifying women who should be

monitored more intensively because they are at higher risk of developing high-grade lesions, especially due to the fact that the positive predictive value of current screening methods in the vaccinated population will decrease[30]. Then the impetus for new screening technologies or advances in the developed world is predominantly driven by the need to increase the positive predictive value of molecular diagnostics of HPV infections and reduce the over-management of low and often transient abnormalities. In these situations, several markers are under investigation.

HPV causes CC. The latest scientific evidence shows that screening for HPV is better than screening for abnormal cytology with the Pap test, so HPV testing is being implemented nationally. The main disadvantage is that more women will test positive and be referred for further diagnostics. Most of these infections are harmless and do not require treatment, and a balance must be struck by identifying women who have H-SIL changes and minimizing the unnecessary referral of anxious women[31] . The Randomized Trials in Screening to Improve Cytology (ARTISTIC) sampled 24,510 women undergoing cervical screening in Greater Manchester in 2001–2003. After 3 and 6 years, the same women were tested for HPV[32]. The patients were then returned for routine screening. They were followed through national cancer screening and registration records until the end of 2015. Comparing HPV results taken at baseline with results collected 3 years later, it was concluded that HPV infections are very persistent. About three-quarters of women with HPV infection but no abnormal cells clear their infections within 3 years. Their risk of developing cancer within 3 years is low (1.5%), so intensive monitoring is unnecessary. Furthermore, 40% of those who remain positive for human papillomavirus (HPV+) have cleared their initial infection, meaning they are also at much lower risk of disease than those with persistent infections[33].

Conclusion

Organized screening is of great importance for reducing the incidence and mortality of malignant tumors, but it is organizationally very demanding, as confirmed by the results obtained so far. It is necessary to continue working on strengthening organized screening for CC in the areas where screening is implemented and to introduce it in all areas of our country where there are conditions for its implementation. There is a plan to implement method-based tools and applications to facilitate the triage of abnormal test results for clinicians and when to refer for colposcopy. In the future, further HPV genotyping and molecular tests may help to better triage women with positive HPV tests to reduce unnecessary referrals for colposcopy. Vaccinated populations will also require less frequent cervical screening.

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