# HPV GENOTYPES COINFECTIONS AND HEALTH RISK - PRELIMINARY STUDY OF THE EAST ROMANIAN POPULATION

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## ABSTRACT

The study aims to identify the degree of infection and co-infection with HPV strains in people of different ages, to assess the risk associated with lack of immunization of the Romanian population. In this study we are looking at the prevalence and relationship of the different types of HPV strains present in the 37 cases with suspected HPV infection that were analyzed, in the period 2018-2019, within the Prodiagnostic analysis laboratory, in Constanța, Romania. Of the total number of people analyzed for the case study, 45.94% (17 out of 37) tested positive for HPV infection. The analysis of the frequency of strains by risk categories shows that the highest percentage was 48% in the case of high-risk strains, followed by the percentage of frequency of strains with unknown risk (44%) and that of low-risk strains (8 %); The analysis by age indicates the maximum infection rate recorded belonging to the age group between 23 and 34 years. The association between strains and the 50% frequency indicates an important aspect of the infection as well as important data for diagnosis and treatment and involves more rigorous monitoring of patients with such associations, the risk increases with the associations.

***Keywords:*** *HPV, co-infections, strains, screening, lesions*

## INTRODUCTION

HPV are double-stranded DNA viruses that infect the stratified epithelium of the skin and mucous membranes. There have been identified approximately 200 strains with potential for induction of transformations. HPV serotypes are differentiated between one another by the genetic sequence of the external L1 protein capsid. Of these, 15 are classified as having high oncogenic risk (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 68, 73 and 82), 3 with probable oncogenic risk (26, 53 and 66) and 12 with low oncogenic risk (6,11, 40, 42, 43, 44, 54, 61, 70, 72, 81, CP6108) [1].

The infection appears to begin with the virus entering through a site of epithelial disruption (microlesion) that allows viral access to the basal epithelial layer. HPV-16 penetration occurs through clathrin-facilitated endocytosis, although other types of HPV may have other mechanisms of cell penetration [2].

Cervical HPV infection with oncogenic risk causes cervical morphological lesions, from normal (normal cytology) to various stages of preneoplastic lesions (CIN 1, CIN 2, CIN 3 / CIS) and invasive cervical cancer. Cervical infection is established by determining HPV DNA in cervical cells by various methods.

Clinically, the most important manifestation of HPV infection in the cervix is considered to be cervical intraepithelial neoplasia (CIN). CIN are cellular lesions with unstable genetic bases, presenting a risk of evolution in extensive cervical cancer, of 30-40%. These untreated lesions, CIN 2 or CIN 3, can cause cervical cancer in a few years or even decades later. The average period of natural evolution to invasive neoplasm is about a few years (12 years) [3].

Certain HPV serotypes more frequently induce oncogenesis, which includes them in the high-risk oncogenic HR-HPV strains, namely serotypes 16, 18 and 45.

These data suggest that in laboratory evaluation, high-risk oncogenic HPV DNA genotyping is a necessary test in suspected lesions and is an important indicator of the risk of developing high-grade or more advanced squamous intraepithelial lesions [4].

Immunohistochemical, cytogenetic and molecular studies [7] have shown that low-risk HPV types do not integrate into the host cell genome, while high-risk types are integrated into the nucleus of epithelial cells in the cervix.

Following integration, the protein product of HPV-16 and 18, proteins E7 and E6 respectively, inactivate the tumor suppressor genes, p53 and the RB-1 gene, allowing uncontrolled cell proliferation. It was possible to document morphological abnormalities in cervical lesions, observing a good correlation with underlying cellular events, by using techniques such as proliferation of cellular antigen (PCNA), expression of p16, p53 and nucleolus organizer region (NOR)[3].

HPV is a necessary cause of cervical cancer, but it is not a sufficient cause. Other cofactors are needed for progression from cervical HPV infection to cancer. Tobacco smoking, high parity, long-term hormonal contraceptive use and HIV co-infection have been identified as established cofactors. Co-infection with *Chlamydia trachomatis* and *Herpes simplex* virus type-2, immunosuppression and certain dietary deficiencies are other likely cofactors [4]. Genetic and immunological host factors and viral factors other than type, such as type variants, viral load, and viral integration, are likely important, but have not been clearly identified [4], [5], [9], [11].

The study aims to identify the degree of infection and co-infection with HPV strains in people of different ages, to assess the risk associated with lack of immunization of the Romanian population.

## MATERIALS AND METHODS

37 cases with suspected HPV infestation were analyzed, in the period 2018-2019, within the Prodiagnostic analysis laboratory, in Constanța, Romania. People were between 23 and 64 years old. The endocervical epithelium was taken and analyzed by HPV DNA detection and genotyping in the specialized laboratory of Matei Basarab Medical Center, Bucharest. The following HPV types were tested:

1. With increased oncogenic risk 14 HR-HPV strains: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68;
2. With low oncogenic risk 3 LR-HPV strains: 6, 11, 42;
3. Other types of HPV: 26.40, 53, 54, 55, 61, 62, 64, 67, 69, 70, 71, 72, 73, 81, 82, 83, 84, IS 39, CP6108.

The results were statistically analyzed and interpreted [12], [13].

## RESULTS AND DISCUSSION

Human papillomavirus (HPV) infection is strongly implicated in the etiology of cervical cancer. High-risk HPV types, most commonly types 16 and 18 and less often types 31, 33, 52 and 58 are present in 70-100% of cervical cancer cases. HPV types 6 and 11 are most commonly found in warts, and mixed HPV types can be found in dysplasia [6].

The analysis of the presence of HPV strains shows that there are 25 different strains, belonging to three categories, taking into account the risk:

1. high-risk HPV strains (61, 52, 31, 56, 66, 16, 51, 39, 58, 18, 35, 59)
2. low-risk strains (6, 42)
3. other strains (81,54, 62, 67, Cp6108, 70, 71, 55, 69, 53, 84).

Of the total number of people analyzed for the case study, 45.94% (17 out of 37) tested positive for HPV infection.

As can be seen in Table 1, most cases indicate that there are several associated strains (co-infection) and with different degrees of risk. It should be noted that 6 of the 17 positive cases analyzed (35.29 %) show the association between 2, 3 or 4 high-risk strains. Another 6 cases show co-infection between high-risk strains and other lesser-known strains (Table1).

***Table 1.*** *Frequency of HPV strains in the group of participants (n = 17) positive and aspects regarding co-infection and frequency of strains (LR = low-risk, HR = high risk)*

| Crt. no. | Strain type | Frequency (%) | Co-infections |
|---|---|---|---|
| | HPV81 | 23.52 | 81, 61, 52, 62 |
| | HPV61 (HR) | 17.64 | 61, 81, 54, 31, 16, 18, 71, 62, 39, 42, 84, CP6108 |
| | HPV 52 (HR) | 29.41 | 52, 54, 62, 31, 62, 35, 59, 55, 69, 35 |
| | HPV54 | 11.76 | 54, 31, 16, 18, 71 |
| | HPV31 (HR) | 17.64 | 31, 56, 66, 16, 18, 71, 62,35,59,55,69 |
| | HPV56 (HR) | 5.88 | 56, 66 |
| | HPV66 (HR) | 5.88 | 0 |
| | HPV62 | 29.41 | 62, 67, 16, 70, 35, 59, 55, 69, CP6108 |
| | HPV CP6108 | 11.76 | CP6108, 62, 67, 61, 39, 42, 84 |
| | HPV67 | 5.88 | 67, CP6109 |
| | HPV16 (HR) | 17. 64 | 16, 70, 39, 58, 18, 71, |
| | HPV70 | 5.88 | 70, 16, 62 |
| | HPV 51 (HR) | 5.88 | 0 |
| | HPV 39 (HR) | 11.76 | 39, 16, 58, 61, 62, CP6109, 42, 84, CP6109 |
| | HPV 58 (HR) | 5.88 | 58, 16, 39 |
| | HPV 6 (LR) | 5.88 | 0 |
| | HPV 18 (HR) | 5.88 | 18, 71, 16, 31, 54, 61 |
| | HPV 71 | 5.88 | 71, 18, 16, 31, 54, 61 |
| | HPV 35 (HR) | 11.76 | 35, 52, 31, 62, 59, 55, 69 |
| | HPV 55 | 5.88 | 55, 35, 52, 31, 62, 59, 69 |
| | HPV 59 (HR) | 5.88 | 59, 55, 35, 52, 31, 62, 69 |
| | HPV 69 | 5.88 | 69, 55, 35, 52, 31, 62, 59 |
| | HPV 53 | 5.88 | 0 |
| | HPV 42 (LR) | 5.88 | 42, 61,81, 54, 31, 16, 18, 71, 62, 39, 84, CP6109 |
| | HPV 84 | 5.88 | 84, 61,81, 54, 31, 16, 18, 71, 62, 39, 42, CP6109 |

As can be seen in the analysis, 21 of the 25 strains (84%) are co-infected, in some cases more than 2 strains being present in the same person (Table 1). These aspects are important for diagnosis and treatment and involve more rigorous monitoring of patients with such associations.

The analysis of the frequency of strains by risk categories highlights the fact that the highest percentage was 48% in the case of high-risk strains, followed by the percentage of frequency of strains with unknown risk (44%) and that of low-risk strains (8 %), Figure 1.

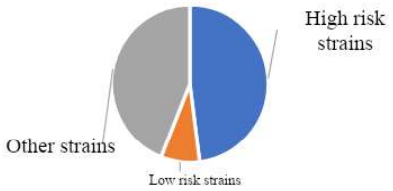


***Fig. 1.*** *Frequency (%) of HPV strains by risk categories, identified in the case of the analyzed group*

Of the total number of strains analyzed, 12 are very high risk (HR-HPV). These strains are found with a frequency between 10- 40%. The most common are HPV52, HPV61, HPV31 and HPV16 from these having 25% frequency (HPV61, HPV31, HPV16) and 41% HPV52, Figure 2.

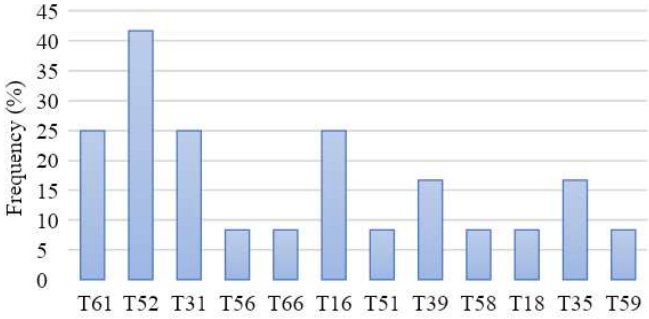


***Fig. 2.*** *Frequency of high-risk HR-HPV strains*

Worldwide, it is generally considered that the most frequently encountered serotypes in patients with CIN 3 or more advanced lesions are HPV 16 and 18. Recent studies have shown large differences in the variation of the serotype 18 prevalence of advanced neoplastic lesions [6], [11].

From the category of strains with unknown or low risk (LR-HPV), only 4 strains (T81, T54, T62, TCP6109) are noted as having a frequency of over 10% (Figure 3).

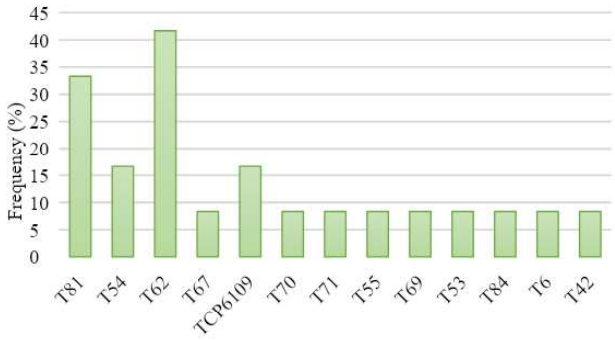


***Fig. 3.*** *Frequency of low-risk (LR-HPV) or unknown risk HPV strains*

The analyzed batch was divided into three classes of age (Fig. 4), a percentage of 25%, the maximum recorded belonging to the group between 23 and 34 years. This result can be associated with conceptual differences in the relationship between partners and education.

There are no known data on the socioeconomic level of people screened or any other factors that would promote the risk of contamination: immunosuppression, smoking, inadequate use of contraceptive methods, infections, sexual behavior, gene polymorphism's [9], [10], [11], [14].

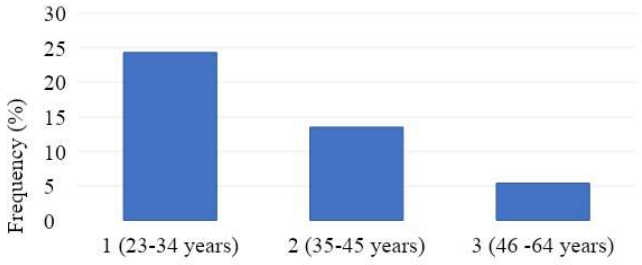


***Fig. 4.*** *Frequency of comparative HPV-positive samples by age classes*

In a similar study from the north Romanian population, prevalence results showed out of the total tested samples, about 37.3% from samples were positive for HPV/DNA. From them 23.3% were single HPV type infections and 14% tested positive for multiple HPV types [6].

The Global strategy towards eliminating cervical cancer as a public health problem adopted by the WHA in 2020, recommends a comprehensive approach to cervical cancer prevention and control. The recommended set of actions should be multidisciplinary[15], including components ranging from community education, social mobilization, vaccination, screening, treatment and palliative care across the life course [9].

Cervical cancer screening involves testing for pre-cancer and cancer, more and more testing for HPV infection is performed. Testing is done among women who have no symptoms and may feel perfectly healthy. When screening detects an HPV infection or pre-cancerous lesions, these can easily be treated, and cancer can be avoided. Screening can also detect cancer at an early stage and treatment has a high potential for cure. Screening has to be linked to the treatment and management of positive screening tests. Screening without proper management in place is not ethical.

The World Health Assembly adopted the global strategy to accelerate the elimination of cervical cancer as a public health problem and its associated goals and targets for the period 2020–2030 [8], [9].

Romania is struggling with a high rate of cervical cancer. The National Screening Program for the early detection of cervical cancer targets a segment of the extended population (about 6 million women). To date, about 700,000 women have benefited from free Babeş Papanicolaou testing services (coverage rate 12%). It is estimated that 49% of all possible cases of cervical cancer have been prevented by population screening.

It is considered that in Romania the information about the risk of cervical cancer is no longer sufficient, and methods of awareness of cervical cancer causes and prevention can reduce risk by 80%, if there is a mass proportion gain [9].

## CONCLUSION

Of the total number of people analyzed for the case study, 45% (17 out of 37) tested positive for HPV infection.

Frequency analysis of strains by risk categories reveals that the largest percentage was recorded in the strains with high risk (48%), followed by the frequency of strains with unknown risk (44%) and that of strains with low risk (8 %).

The analysis by age class indicates the maximum of recorded strains belonging to the age group between 23 and 34 years. This result can be associated with conceptual differences in the relationship between partners and education.

In our study the most common high-risk serotypes were HPV61, HPV31, HPV52, and HPV16. These strains are found with a frequency between 10 - 40%. The most common are HPV52, HPV61, HPV31 and HPV16 having the values of frequency at 25% (HPV61, HPV31, HPV16) and 41% respectively (HPV52).

The association between strains and the 45% frequency indicates an important aspect of the infection as well as important data for diagnosis and treatment and involves a more rigorous monitoring of patients with such associations, the risk increases with the associations.

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