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Original Article

PREVALENCE OF HPV16 IGG ANTIBODY AND RT-PCR DNA-DETECTION IN PATIENTS WITH LARYNGEAL CARCINOMA AND CERVICAL DYSPLASIA

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Summary

Human papillomaviruses (HPVs) are associated with the most common sexually transmitted infections. It is well documented that high-risk (HR)-HPV types are etiologically associated with some cancers. The aim of the study was to investigate HPV16-DNA positivity and prevalence of IgG antibody against HPV16 in patients with laryngeal carcinoma and precancerous lesions of cervix uteri in Pleven region, Bulgaria. Material/Methods: We performed a cross-sectional study and investigated clinical materials. Attached is real-time PCR-analysis for detection of HPV16-DNA. HPV-specific antibody response by enzyme-linked immunosorbent assay (ELISA) test for detection and quantification of specific IgG antibodies in serum were used. Results: For the six- month period, 30 samples were collected and tested. Fourteen of them were found in patients with carcinoma of the larynx and sixteen – in patients with various lesions of cervix uteri. We found that six patients (42.8%) in the first group and eight patients (50%) in the second group were HPV16-DNA-positive. Different age groups were affected. The sera analyzed in this study showed that seven patients (50%) with carcinoma of the larynx were seropositive of whom four (57%) were males. Fourteen of the females with dysplasia (88%) were seropositive. Matching DNA positivity and antibody response were found in 29% of the patients with laryngeal cancer. The match was found in 50% of the females with cervical dysplasia. Conclusions: Real-time PCR is a rapid, cost-effective method for detection of HPVs. A high level of seropositivity was found in the two groups of patients.

Key words: HPV, prevalence, HPV16-DNA-detection, prophylaxis

Introduction

It is assumed that Human papillomavirus (HPV) is related to the most common sexually transmitted infections. Despite its wide distribution, the changes observed in the majority of infected individuals are benign. Development of malignancies in various sites in the body is also a problem associated with

HPV. In the 1980s, a connection between HPV viruses and cervical cancer was proved. Later, an association was found with cancer of the larynx. There are modern methods for diagnosing and typing viruses and over 100 types of HPVs with oncogenic potential are known today, and HPV 16, 18, 30, 31, 33, 45, 52, etc. are most often quoted. Studies in Europe, including Bulgaria, proved the highest percentage of presence of HPV 16 and 18. There is an increase in the number of infected women with cervical cancer, and laryngeal cancer affects mostly men [1-3]. Atypical and subclinical infections may mean late diagnosis and treatment. Many specialists have approached these issues. After the development of vaccines on the agenda are immunoprophylaxis, early diagnosis, and prevention. Public health might benefit from seroepidemiological population surveys that identify risk groups [4]. Comparison of these surveys could help with designing screening and prevention programs to reduce morbidity and mortality from cancers associated with HPV. Reliable vaccines are available to prevent these diseases. In January 2008, a panel of European Centre for Disease Prevention and Control (ECDC) issued a Guidance for introducing HPV vaccines in EU countries. Two vaccines were licensed in Bulgaria: bivalent (types 16 and 18) and quadrivalent (types 6, 11, 16 and 18). Since 2012, for girls age 13, a two-dose vaccination has been introduced. The aim of this study was to investigate an age-specific HPV16 prevalence in patients and to identify the most affected groups of the population.

Materials and Methods

This study is a part of a scientific research project. Informed consent was signed by all the patients included. The study was reviewed and approved by the ethics committee for clinical research at the Medical University – Pleven. We performed a cross-sectional study and investigated clinical materials.

Patients

We studied 30 patients, divided into two groups. The first group included fourteen patients treated in the Clinic of Otorhinolaryngology, University Hospital – Pleven – 11 (79%) males and 3 (21%) females, mean age 61 (range 23-77). Their clinical diagnosis was carcinoma of the larynx. The histological diagnosis of all patients in this group was laryngeal squamous cell carcinoma. The second group included sixteen women, treated at the Gynecologic Oncology Clinic, University Hospital – Pleven. The mean age of the patients was 44 (range 27-72). Histological analysis showed that HPV16-DNA-positive females from this group had cervical dysplasia – cervical intraepithelial neoplasia (CIN I and CIN II) and carcinoma in situ (CIS).

Serological survey

We collected and analyzed sera from the two groups. Samples were stored and treated after manufacturers' recommendations. For qualitative determination of HPV type 16, Human papillomavirus type 16 L1-capsids (HPV16L1) antibody (IgG) ELISA kit (CUSABIO®) was applied.

DNA-analysis

Biopsy specimens were collected from patients operated on. Real-time PCR-analysis for detection of HPV16-DNA was performed. DNA-sorb-C nucleic acid extraction kit AmpliSens®-US and Primerdesign™ Ltdgenesig®-UK Human Papillomavirus 16-E6 gene genesig® Advanced Kit were used. Data analysis and processing were made with Microsoft Excel 2003.

Results

Distribution of the patients from the Clinic of Otorhinolaryngology according to survey results is shown in Figure 1.

We found that six patients (42.8%) were HPV16-DNA positive. Four of them were positive for IgG antibody against HPV16. In those negative for viral DNA, serological tests showed the presence of IgG antibody in three patients and its absence in five patients. Age distribution of patients according to the results is shown in Figure 2.

Distribution of the patients from the Clinic of Gynecologic Oncology according to test results is shown in Figure 3.

Age distribution of patients according to survey results is shown in Figure 4.

Figure 5 shows the results from the histological investigations for the second group.

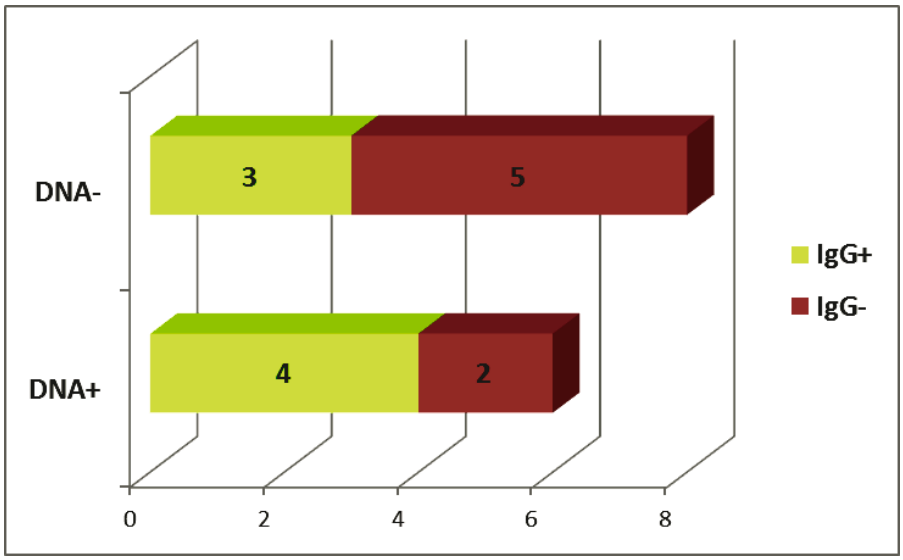

Figure 1. HPV16-DNA-detection and prevalence of HPV16 in patients with laryngeal carcinoma

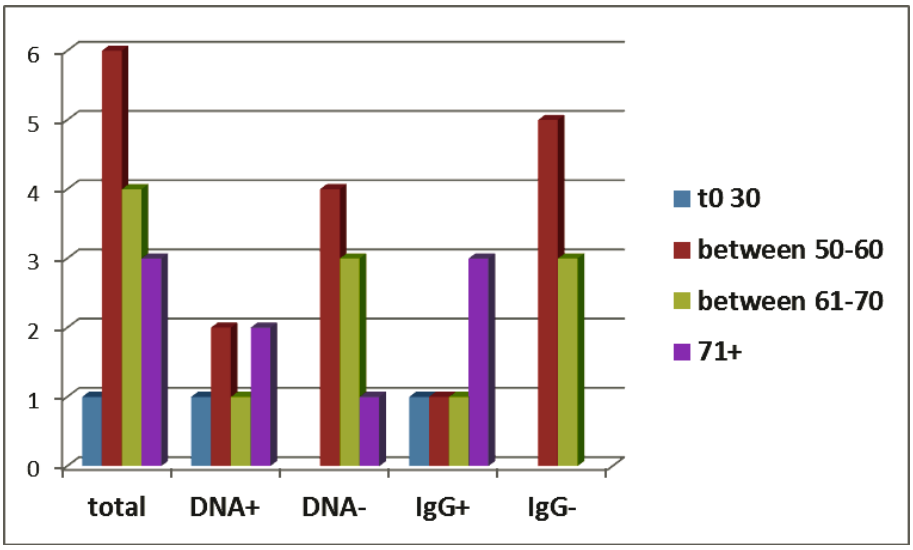

Figure 2. Age distribution of patients with laryngeal carcinoma according to test results

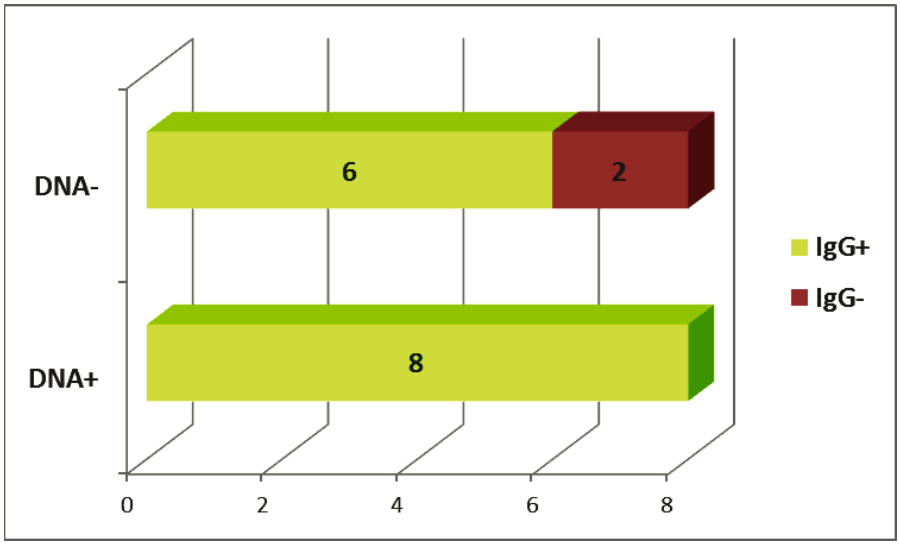

Figure 3. HPV16-DNA-detection and prevalence of HPV16 antibody in patients with cervical dysplasia

Discussion

This study showed high-level viral prevalence in the investigated groups of patients. HPV16 is associated with increased risk of cervical and oropharyngeal carcinoma. Diagnosis of infection caused by oncogenic types of HPVs is of great importance for prevention, early detection and proper treatment of affected patients. In this sense, the methods applied in this study (RT-PCR and ELISA) are current, specific, credible and consistent with research in other countries [5].

During the last years, the incidence of laryngeal carcinoma in Bulgarian men is 3.4%. The disease is a problem for older men but as our research has shown, it affected women too. We found that in patients with cancer of the larynx a 42% presence of viral particles were found. Similar results have been reported by other authors; 50% of those surveyed had specific IgG antibodies. Their presence indicates exposure to the virus with a different prescription. The average age of the patients with laryngeal carcinoma is 61. The most affected age group is between 50 and 60 years. Positive results with both methods were found in four patients – two women and two men. Although clinically manifested cases are more common in men, we believe that both sexes are equally susceptible to the virus. Other risk factors such as smoking, alcohol abuse, and others can contribute to pathological cell transformation and cancer development in men [6].

HPV is associated with the most common sexually transmitted infections worldwide. Sexually transmitted HPV types fall into two categories: low-risk HPVs, which do not cause cancer but can cause skin warts, and high-risk HPVs, which can cause cancer [7]. Responsible for tumor transformation are types 16, 18, 31, 33, etc. It is known that types 16 and 18 are etiologically associated with cervical carcinoma. The presence of the virus in precancerous lesions is also worth considering: the second group included women with varying degrees of dysplasia. The results showed that 50% are HPV16-DNA- positive, and all were HPV16 IgG positive. Of the women HPV16-DNA-negative, six (75%) had HPV16 IgG antibody. The mean age of women was 44 (range 27-72), and the age group most affected was that of the 40-50-year old. Histological examination revealed the presence of HPV16 in women with different grades of cervical dysplasia [8]. It is appropriate to discuss immunization for high-risk groups. Targeting slightly older girls and men and young women with catch-up vaccination is likely to decrease the incidence of cancer in these groups.

Conclusions

Despite the small number of patients studied, high prevalence and presence of viral DNA was found, which could be indirectly interpreted as a reason for the occurrence of malignancies [9, 10]. The documentation of the causative organism and the establishment of prevalence in the population could help identify risk groups and build a strategy for preventive measures against them.

An important element of prophylaxis of HPV-related cancers is the implementation of vaccines, which could lead to solving this problem of public health.

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