Prevalence of Human Papillomavirus in Laryngeal Squamous Cell Carcinoma in Azerbaijan Population

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Abstract

Objective of the study: There is enough evidence for the carcinogenicity of HPV16 type in the head and neck cancers. This study aimed to address key information gaps of the prevalence of HPV infection in laryngeal squamous cell carcinoma in Azerbaijan population.

Materials and methods: 50 LSCC pretreatment FFPE biopsy tumor materials, 10 cervical cancer FFPE samples and 38 fresh pretreatment LSCC tumor materials were analyzed using RT-PCR technology and Anyplex II HPV28 genotyping assay. The FFPE tumor material with HPV positive history from cervical cancer was as control in this study.

Results: DNA extraction from FFPE and fresh samples was successful for all 98 tumor materials. The results of HPV genotyping of cervical cancer patients (n=10) with Anyplex II HPV28 assay detected 8 HPV16-positive cancers, 1 samples with HPV18-positive and 1 cancer material with HPV16+HPV18+HPVS8. The results were predictable, because in these patients, HPV positivity was validated by Pap smear method. Based on this, we expect to get true information about HPV-related LSCC. Anyplex IIHPV28 genotyping assay was detected only one HPV positive sample of 61 LSCC: it was low-risk cancer-related HPV54 subtype in 47 age’s patient with 20 years tobacco and alcohol consumption history. High-risk cancer related HPV16 and HPV18 subtypes have no detected in analysis patients.

Conclusion: Thus, we suggested that PCR techniques and Anyplex II HPV28 genotyping assay were available to detect HPVs from fresh LSCC material. Our results support us to believe that Azerbaijan belongs to HPV low-incidence geographic region of laryngeal squamous cell carcinoma patients, but large-scale population-based studies are needed to confirm our findings.

Keywords: Laryngeal squamous cell carcinoma; High-risk HPV types; Low-risk HPV types; HPV detection molecular technology; HPV low-incidence geographic region

Abbreviations: HPV: Human papillomavirus; LSCC: Laryngeal squamous cell carcinoma; HNSCC: Head and neck squamous cell carcinoma; FDA: Food and Drug Administration; FFPE: Formalin-fixed, paraffin-embedded.

Introduction

The majority of head and neck cancers are associated with high tobacco and alcohol consumptions. However, the certain high-risk type of HPV infection has been suggested as etiological factors, which associated with head and neck cancers [1-6]. The International Agency for Research in Cancer (IARC) has concluded certain points on the carcinogenicity of HPV in humans. These are (a) there is enough evidence for the carcinogenicity of HPV type 16 in the oral cavity, oropharynx (including tonsil cancer, base of tongue cancer) and other oropharyngeal cancer sites, and (b) limited evidence for laryngeal cancer [7].

HPVs are exclusively intraepithelial pathogens and they infect and replicate only in fully differentiating squamous epithelium [8] and HPV infection was found just in head and neck squamous cell carcinoma across the studies obtained [1,2,4,5]. The meta-analysis results indicate that the reported wide variability in HPV detection rates in HNSCC is not due the HPV detection techniques, but it is explained by the geographic origin of the study. These data substantiate the recent elaborated concept that HNSCC might have a different etiology in low-incidence and high-incidence geographic regions; HPV is the second most important factor [9-11].
Different detection method technologies are applied to tissue HPV genotypes analysis, but there is no Food and Drug Administration approved molecular tests to detect HPV infection in HNSCC [12–15]. There are 193 distinct molecular tests that are commercially available on the global market for the detection of HPV in cervical specimens. 110 of the 193 (57%) available tests have a least one publication in peer-reviewed literature [16–20]. The studies indicated that any of the commercially available tests approved for cervical cytology could be used by clinical laboratory HPV detection in oropharyngeal biopsy specimens [20]. A recent comparison of the Roche Cobas 4800 HPV Real-time PCR test with standard methods of in situ hybridization (ISH) for high-risk HPV and immunohistochemistry (IHC) for HPV16 on HNSCC specimens demonstrated a sensitivity of 100% and a specificity of 86% for the Cobas system and this molecular technology detection method were recommended for HNSCC HPV’s screening [18]. In Azerbaijan, this is the first study that evaluated HPV infection prevalence in the HNSCC. This study aimed to address key information gaps of the prevalence of HPV infection in laryngeal squamous cell carcinoma in Azerbaijan population.

**Materials and Methods**

50 laryngeal squamous cell carcinoma pretreatment FFPE biopsy tumor materials with stage I-IV, 8 HPV16 and 2 HPV18 positive cervical cancer FFPE samples and 38 fresh pretreatment LSCC tumor materials were included in study. All patients addressed to National Center of Oncology from 2011 to 2017 and cancer diagnoses were validated by pathomorphology. HPV16 (8 women) and HPV18 (2 women) in cervical cancer materials were approved by cytology method in advance. The larynx cancer specimens evaluated from 57 males and 31 females with an average age from 40 to 66 years. 45 of 98 patients had smoking histories and 23 had alcohol consumption histories. 37 patients had both smoking and alcohol histories. Thus 60 formalin-fixed, paraffin-embedded tumor materials and 38 fresh pretreatment biopsy materials were used for DNA isolation analysis.

Genomic DNA was extracted from FFPE tumor core and fresh tumor material using DNeasy Blood and Tissue kit (Qiagen). Quality and quantity of DNA was estimated spectrometrically (NanoDrop Technologies). For each test 5 μL (50 ng) DNA material was used. For HPV genotyping were used AnyplexTM II HPV28 genotype detection assay (AnyplexTM II HPV28 genotype detection, Seegene, Southern Korea) and Real-Time PCR condition as descript [13]. Analyses were conducted by CFX 96 Real-Time System (Bio-Rad, US).

**Results**

In this study, total 60 clinical FFPE and 38 fresh tumor samples were analyzed with AnyplexTM II HPV28 assay. This kit has confirmed the cervical FFPE sample for HPV genotyping [13] as a reference to the PCR protocol. AnyplexTM II HPV28 detects 28 human papillomavirus genotypes: 19 high-risk HPV types and 9 low-risk HPV types. The present day this genotyping assay considered concordant with other commercial assays for HPV detection, with comparable sensitivity and specificity for ≥CIN2 (cervical intraepithelial neoplasia grade 2 or higher) detection [16]. The FFPE tumor materials with HPV16/18-positive history from cervical cancer were as control in this study. DNA extraction from FFPE and fresh samples were successful for all 98 tumor materials. Genetic materials quality and quantity were suitable for analysis condition.

The results of HPV genotyping of cervical cancer patients (n=10) detected 8 HPV16-positive cancers, 1 samples with HPV18-positive and 1 cancer material with HPV16+HPV18+HPV58. It was expected results of genotyping analyzing cervical cancer patients, because cytology method already validated HPV history in these materials. Since it is known that the microenvironment of laryngeal mucosa is similar to that of the uterine cervix which has an epithelial junction area between squamous and columnar epithelia; the junction area is a potential site for HPV infection [5,21]. Based on this information we expect to get true information about HPV status in laryngeal squamous cell carcinoma material using PCR-technology and AnyplexTM II HPV28 genotyping assay.

In spite of DNA extracted from 50 FFEP laryngeal squamous cell carcinoma materials that were enough for manage RT-PCR reaction we could not get genotyping results in 23 (46%) patients. AnyplexTM II HPV28 genotyping assay had success in 27 (54%) patients with LSCC and only one of 27 laryngeal squamous cell carcinoma samples that archived as FFPE material has positive HPV reaction: it was low-risk cancer-related HPV54 genotype in 47 age patient with larynx T3NOM0 cancer history. He was admitted to National Center of Oncology in 2011 and had a total laryngectomy. In other 26 FFPE samples high-risk cancer-related HPV16 and HPV18 subtypes have no detected.

38 genetic materials (DNA) were isolated from fresh laryngeal squamous cell carcinoma that immediately after surgery was put unto 0.05% PB buffer and delivered to laboratory. Genotyping analysis results had success for all 38 patients. No HPV infection was detected in LSCC fresh materials. Thus, AnyplexTM II HPV28 genotyping result from fresh LSCC materials (n=38) and FFPE fixed cervical cancer materials (n=10) had full success for HPV status definition, what cannot be said about FFPE fixed LSCC materials: in this case 46% AnyplexTM II HPV28 genotyping assay results were invalid.

In this study using wide spectrum high and low cancer-related HPV genotypes primer have got preliminary information about PCR technology detection and prevalence of HPV infection in laryngeal squamous cell carcinoma in Azerbaijan population. We tried to find the research study conclusion in our close neighbors’ population of HPV prevalence in head and neck squamous cell carcinoma: in Turkey it was only one research result about PCR amplification that had success in 61 of 77 HNSCC patients. Among the 61 HNSCC patients only 3 patients were HPV positive (4.9%). HPV 16 subtype was detected in one patient who was 70 years old male, stage III laryngeal cancer with a smoking history. The
subtypes detected in other two patients were different from 16 and 18. One of these patients was 42 years old nonsmoker female stage IV hypopharyngeal cancer and the other one was 56 years old smoker male with stage II oropharyngeal cancer [22]. Authors suggested a low prevalence of HPV in Turkish HNSCC patients, while currently, HPV-positive and HPV-negative oropharyngeal squamous cell carcinoma (OPSCCs) are thought to be two distinct diseases in Europe and the United States [23]. In Iranian population, authors conducted retrospective study from 2011-2017 for survey HPV occurrence in HNSCCs as part of a comprehensive molecular epidemiology approach [24]. A total of 156 FFPE blocks were collected. HPVs genotyping analyses were conducted using three methods. Overall, 5/156 (3.2%) patients (3 females and 2 males) were found to be HPV positive. HPV genotyping revealed HPV types 16, 2, 27, and 43 in these malignancies. Although majority authors suggested that high risk HPV genotypes have been associated with HNSCCs, this study finding indicated a potential of low risk HPV types to also contribute to such malignancies.

We believe that there are currently no molecular tests and molecular methods that have been approved by the FDA for the detection of HPVs infection in HNSCC, and despite numerous studies on cancer that are associated with high-risk HPV genotypes, this problem remains open in the HNSCC.

The archived formalin-fixed, paraffin-embedded (FFPE) tissue samples can be invaluable resources for profiling HPVs genotyping in HLSCC, but in our study fresh tumor material was more effective. We think FFPE tissue can be a difficult task due to the damaged template that results from the archiving process. Because the high-oncogenic risk type HPV can induce tumorigenesis via the E6 and E7 viral oncoproteins [25], and these oncoproteins can functionally inactivate the tumor suppressor proteins p53 and pRB, resulting in a loss of cell cycle regulation [26,27] for forward actions we have to get more and detailed information about HPV-positive HNSCC.

Conclusion

Thus, this is the first study that evaluated HPV prevalence in laryngeal squamous cell carcinoma in Azerbaijan and we suggested that fresh tumor material and any HPV genotype detected PCR techniques may be available for this location.

Our results support us to believe that Azerbaijan belongs to HPV low-incidence geographic region of laryngeal squamous cell carcinoma patients, but large-scale population-based studies are needed to confirm our findings.

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