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Original article

Retrospective analysis of oncogenic human papilloma virus and Epstein-Barr virus prevalence in Turkish nasopharyngeal cancer patients☆

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ABSTRACT

Nasopharyngeal carcinoma (NPC) is associated with the Epstein-Barr virus (EBV). Human papilloma virus (HPV) has also been detected in NPC cases. In this retrospective study, we analyze the frequency of EBV and HPV infection in 82 Turkish patients with NPC.

A total of 82 were evaluated for EBV and HPV. *In situ* hybridization (ISH) was performed for EBV. HPV-ISH and P16 immunohistochemistry used to determine the HPV status.

Seventy-two of the 82 (87%) NPC patients were EBV-positive. The highest rate of EBV-positivity was found in undifferentiated NPC patients, which accounted for 65 of 68 (95.6%) undifferentiated cases. One of the 82 NPC patients whose tumor was non-keratinizing differentiated, contained HPV.

Our data shows that EBV is closely associated with NPC in Turkey. We found lower rates of HPV-positivity in NPC patients than in North American populations. In addition, both EBV and HPV-negativity were more associated with decreased survival than EBV-positive cases.

1. Introduction

Nasopharyngeal carcinoma (NPC) is the most common type of carcinoma arising in nasopharyngeal mucosa that shows squamous differentiation. NPC constitutes 0.6% of all cancers worldwide but there are wide geographical and ethnic variations in its rates [1]. Compared to the rest of the world, NPC incidence is considerably higher in southern China, Southeast Asia, the Middle East and North Africa. The incidence is highest in South-Eastern Asia (6.5 per 100.000 for males, 2.8 per 100.000 for females), and the incidence is lowest in central America (0.2 per 100.000 for males, 0.1 per 100.000 for females) [1]. Within these populations, there is remarkable heterogeneity among ethnic lines [2].

The heterogeneity of NPC rates depends on viral, environmental and genetic components of multifactorial etiology. NPC is endemic in the Far East and is associated with Epstein-Barr virus (EBV). The strong association of EBV with NPC indicates a probable oncogenic role for the virus in the genesis of this tumor. Although EBV has long been implicated in nasopharyngeal carcinogenesis, other potential etiological factors have yet to be fully addressed. High levels of volatile nitrosamines in food, salted fish, cigarette smoking, formaldehyde exposure and prior radiation exposure are currently the most widely implicated environmental factors [3–7]. Elevated risk prevails despite migration from high to low risk areas, depending on the country of origin [8]. Ethnicity, genetic predisposition, EBV and lifestyle are the principal reasons for the increased risk in migrants. Significant associations between elevated NPC risk and rural household type, low socioeconomic status, fried food con-

Abbreviations: NPC, Nasophanryngeal carcinoma; ISH, *In situ* hybridization; EBV, Epstein-Barr virus; HPV, Human papilloma virüs; EBER, Epstein-Barr virus-encoded small RNA.

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Table 1
Patient and tumor characteristics of NPC cases.

Characteristics	All patients(n = 82)	EBV positive(n = 72)	HPV positive(n = 1)	Both negative(n = 9)
Age				
Median	52	51	65	58
Range	11–78	11–77	–	27–78
Gender, n (%)				
Male	63 (76.8)	55 (76.4)	1 (100)	7 (77.8)
Female	19 (23.2)	17 (23.6)	0 (0)	2 (22.2)
WHO type, n(%)				
Keratinizing	4 (4.9)	2 (2.8)	0 (0)	2 (22.2)
Nonkeratinizing-Diff.	10 (12.2)	7 (9.7)	1 (100)	2 (22.2)
Nonkeratinizing-Undiff.	68 (82.9)	63 (87.5)	0 (0)	5 (55.6)
Prognosis, n(%)				
Alive	49 (59.8)	47 (65.3)	–	2 (22.2)
Died	33 (40.2)	25 (34.7)	1 (100)	7 (77.8)
EBV, n(%)				
Positive	72 (87.8)	72 (100)	–	0 (0)
Negative	10 (12.2)	–	1 (100)	9 (100)
HPV, n(%)				
Positive	1 (1.2)	0 (0)	1 (100)	0 (0)
Negative	81 (98.8)	72 (100)	–	9 (100)
P16, n(%)				
Positive	2 (2.4)	1 (50)	1 (100)	0 (0)
Negative	80 (97.6)	1 (50)	–	0 (0)

sumption, smoking and poor oral hygiene have been reported in the Turkish population [9]. The incidence of NPC in Turkey is 0.6 per 100.000 for males and 0.2 per 100.000 for females [1]. In addition, EBV has been to be associated with NPC in Aegean Turkish patients [10].

Human papilloma virus (HPV) is an epitheliotrophic oncogenic virus that has been detected in a variety of head and neck tumors, especially oropharyngeal carcinomas. Recent significant reports have identified an association of oncogenic HPV with a sub-group of NPCs [11–14].

The remarkable rise of oropharyngeal carcinoma has been attributed to HPV, however, little is known about the prevalence and clinical significance of the virus in NPC [15]. There are some reports about the geographic and racial characteristics of HPV in NPC based on the hypothesis of the contribution of HPV to the pathogenesis of NPC [13,16,17]. The aim of this retrospective study was to determine the prevalence of EBV and oncogenic HPV in NPC using archived tissue from two centers in Turkey.

2. Materials and methods

2.1. Patients

Patients presented to two medical centers in Turkey (Department of Pathology at Hacettepe University and Department of Pathology at Ataturk Education and Research Hospital) were identified retrospectively from pathology databases over a period of 5.5 years (October 2006 to May 2012). Only tumors confirmed to have originated within the nasopharynx were included in this study. Formalin-fixed paraffin embedded (FFPE) tissues were available from 82 patients, of whom 63 were males and 19 females (M/F ratio: 3.3) with a median age of 52 years (range: 19–78 years-old). Hematoxylin and eosin stained slides of all specimens were reevaluated by three pathologists to confirm the diagnosis, identify a WHO grade and select corresponding blocks of primary tumor.

2.2. World Health Organization grading

Hematoxylin-eosin stained whole sections of tumors were graded by three pathologists according to the 2005 World Health Organization (WHO) histologic classification system for NPCs. WHO has classified NPC into keratinizing squamous cell carcinoma (KSCC, formerly WHO type I); non-keratinizing (NK) carcinoma, including differentiated (NK-D, formerly WHO type II) and undifferentiated variants (NK-U, formerly WHO type III); and basaloid squamous cell carcinoma (BSCC) [18].

2.3. EBV detection

To analyze the prevalence of latent viral infection among Turkish patients with NPC, *in situ* hybridization (ISH) for EBV-encoded small RNA (EBER) using the inform EBER probe (1-DNP) was performed on formalin-fixed, paraffin-embedded tissue sections on an automated platform (Ventana Benchmark GX, Tucson AZ). For EBER a positive hybridization was defined as strong diffuse signals in the nucleus of nearly all of the tumor cells. Positive (known EBV-positive NPC) and negative controls were tested together with the tumors of the patients.

2.4. HPV detection

The patients' HPV statuses were assessed by both ISH and p16INK4a immunohistochemistry for all 82 patients. Tumor samples were evaluated by immunohistochemistry (IHC) for expression of the CDK inhibitor p16, a HPV E7 oncoprotein activity biomarker. Overexpression of p16 is observed when Rb is inactivated by the E7 oncoprotein of oncogenic HPV-types.

Immunohistochemical analysis for the p16 was performed on four-micrometer tissue sections per patient. After deparaffinization, rehydration, peroxide blocking, heat retrieval and protein blocking, tissue sections were incubated with a mouse monoclonal antibody (Biocare Medical, dilution 1:200) according to manufacturer's instructions. Slides were then developed using chromogen DAB and counterstained with hematoxylin. The intensity and proportion of tumor cell staining was scored by a single pathologist. We defined p16 positivity as 70% or more tumor cells with strong and diffuse nuclear or nuclear and cytoplasmic p16 expression.

ISH using INFORM HPV III Family 16 Probe was performed on formalin-fixed, paraffin-embedded tissue sections on a Ventana Benchmark automated platform. The INFORM HPV III Family 16