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High-risk HPV Does not Appear to be an Important Risk Factor for Sinonasal Carcinomas in Turkish Population: A Tertiary Center Experience

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Abstract

Background. The sinonasal tract is the second most common site of human papillomavirus (HPV)-related carcinomas in the head and neck. Published data on the association between sinonasal tumors and HPV are quite inconsistent among different regions. *Material and methods*. We performed high-risk HPV DNA in situ hybridization (ISH) and p16 immunohistochemistry on sinonasal carcinomas diagnosed between 2006 and 2016. *Results*. Of 105 sinonasal carcinomas, we found only two (2%) HPV-positive cases; both had non-keratinizing morphology and were diffusely positive for p16. By histologic type, HPV DNA positivity rate was 14% in non-keratinizing squamous cell carcinomas, and we did not detect HPV DNA in any other type of sinonasal carcinomas. Thirteen HPV-negative tumors (7 salivary gland carcinomas, 3 sinonasal undifferentiated carcinomas, 2 keratinizing squamous cell carcinomas, and 1 non-keratinizing squamous cell carcinoma) were positive for p16. In nine carcinomas arising from an underlying sinonasal papilloma, p16 and HPV DNA ISH were evaluated in both carcinoma and papilloma areas and all were negative. Follow-up information was available for 104 patients; 46 (44%) were alive and 58 (55%) died of disease. One of the two HPV-positive patients died of the disease; the other was alive at 100 months of follow-up. *Conclusions*. We detected a much lower percentage of HPV positivity in sinonasal carcinomas when compared to the literature. We believe that our results support various rates of HPV-related carcinomas depending on the geographic and ethnic characteristics.

Keywords

human papillomavirus, sinonasal carcinomas, in situ hybridization, p16, sinonasal papillomas

Introduction

In the past several decades, high-risk human papillomavirus (HPV) infection has been associated with etiopathogenesis of head and neck carcinomas, and its prevalence particularly depends on the anatomic location.[1] The oropharynx is the most common site of origin with up to 80%.[2,3] HPV-related oropharyngeal squamous cell carcinomas arise in younger patients. They are less associated with cigarette and alcohol assumption, display non-keratinizing morphology, and have better overall survival.[2,3] The sinonasal tract is the second most affected site of HPV-related carcinomas; however, unlike the oropharynx, its prognostic significance has been debated.[4–8]

Viral molecules (DNA, E6/E7 mRNA) or cell proteins (p16) can be used as target molecules for the detection of HPV.[9] P16 is a cell cycle protein, which slows down the cell cycle by inhibiting hyperphosphorylation of the retinoblastoma protein (pRb). In HPV-infected cells, HPV E7 oncoprotein inactivates pRb and leads to accumulation of the p16 protein in response. In oropharyngeal squamous cell carcinomas with typical morphology, p16 overexpression by immunohistochemistry is accepted as sufficient evidence for HPV association.[1,10] Although p16 immunohistochemistry is highly sensitive, cheap, and easy to apply, its specificity to detect high-risk HPV in sinonasal carcinomas is relatively low.[1,9,11] For non-oropharyngeal carcinomas with p16 overexpression, additional HPV-specific testing, such as in situ hybridization (ISH) or polymerase chain reaction (PCR), is recommended.[1]

The published data on sinonasal tumors and HPV are quite heterogeneous and diverse depending on the geographic region, study cohorts, HPV genotypes, and HPV

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detection methods.[12,13] Therefore, we here aimed to focus on the presence of high-risk HPV in carcinomas of the sinonasal tract, including salivary gland type carcinomas. To the best of our knowledge, this is the first study from Turkey analyzing high-risk HPV in sinonasal carcinomas by using HPV DNA ISH and p16 immunohistochemistry.

Materials and Methods

Carcinomas primarily originated from the nasal cavity or the paranasal sinuses diagnosed in Istanbul University, Istanbul Faculty of Medicine, Department of Pathology between 2006−2016 were studied. Tumors arising from the vestibular area or the oral cavity were excluded.

We reviewed all hematoxylin and eosin-stained slides and revised the diagnoses of cases according to the 2017 World Health Organization Classification of Head and Neck Tumors. In some instances, immunohistochemical or EBER ISH findings were considered for the differential diagnosis. The clinical information such as age at the diagnosis, sex, and overall survival data were gathered from the electronic medical records.

Immunohistochemistry and In Situ Hybridization

A selected representative paraffin block for each case was cut into 3 – 5 micron-thick sections for ancillary studies. Only non-decalcified samples were used to avoid false-negative results. In cases associated with a sinonasal papilloma, p16 immunohistochemistry and HPV DNA ISH were performed on samples containing both papilloma and carcinoma areas, and the positivity was evaluated separately.

For immunohistochemistry, sections were immunolabeled with an antibody against p16 (mouse monoclonal antibody; clone G175 – 405, BD Biosciences, San Diego, CA; dilution: 1/10; incubation time/temperature: 60 min./ 37 °C; antigen retrieval: EDTA, pH: 8.0) using a Ventana Benchmark XT, IHC/ISH automated staining platforms, Roche diagnostics Ltd, (Arizona, USA). P16 expression was evaluated according to the percentage of stained cells, the intensity of the staining (weak or strong) and the expression pattern (nuclear and/or cytoplasmic). Diffuse-strong cytoplasmic and nuclear staining in ≥70% of the tumor cells was considered positive.

Ventana Inform HPV III Family 16 Probe (B) kit (Ventana Medical Systems, Tucson, AZ) containing a cocktail of genomic probes targeting HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66 genotypes was used for high-risk HPV DNA detection. After deparaffinization, sections were conditioned using citrate buffer and digested with proteinase K. For the staining procedure, in situ hybridization iView Blue Plus Detection Kit reagents, probes, red counterstain II and HybReady solution were loaded into the reagent tray and placed into the Ventana Benchmark XT, IHC/ISH automated staining platform, Roche diagnostics Ltd, (Arizona, USA). Then, the dehydration procedure was proceeded manually as described in the instruction in use.

The results were interpreted using a light microscope (Olympus BX51TF) and the discrete, dot-like nuclear blue hybridization signals in tumor cells were considered indicating an HPV positive tumor. If p16 expression was observed in more than 40% of the tumor cells, ISH was repeated on another appropriate paraffin block.

Two cores of 5 mm in diameter from pulmonary metastases of a cervical squamous cell carcinoma and an oropharyngeal squamous cell carcinoma were embedded in a paraffin block side by side and were used as positive controls. Non-tumoral sinonasal mucosa served as a negative control.

Systematic Literature Review

To document the high-risk HPV status among sinonasal carcinomas from different regions, we searched the English literature via PubMed for studies published between January 2000 and December 2021 using the

Table 1. The Clinicopathologic Findings of Sinonasal Carcinomas Associated with Sinonasal Papilloma.

Case	Sex	Age	Localization	Type of papilloma	Histological type of tumor	Synchronous / Metachronous
1	Male	62	Maxilla	Inverted papilloma	Non-keratinizing SCC	Metachronous
2	Female	70	Nasal cavity	Inverted papilloma	Non-keratinizing SCC	Metachronous
3	Female	78	Maxilla	Inverted papilloma	Non-keratinizing SCC	Synchronous
4	Male	53	Nasal cavity	Inverted papilloma	Verrucous carcinoma	Synchronous
5	Male	71	Maxilla	Inverted papilloma	Keratinizing SCC (well differentiated)	Synchronous
6	Male	63	Nasal cavity	Inverted papilloma	Keratinizing SCC (moderately differentiated)	Synchronous
7	Male	44	Nasal cavity	Inverted papilloma	Keratinizing SCC (poorly differentiated)	Synchronous
8	Male	63	Nasal cavity	Inverted papilloma	Keratinizing SCC (poorly differentiated)	Synchronous
9	Male	36	Maxilla	Inverted papilloma	Small cell carcinoma	Synchronous

SCC; squamous cell carcinoma.

following terms [(sinonasal) AND (HPV)]. Meta-analyses, review articles, studies with <10 cases, and case series of HPV-related multiphenotypic sinonasal carcinomas were excluded. The full texts of the articles including ≥10 sinonasal carcinomas tested for high-risk HPV were reviewed to provide information on the study year, country, HPV testing method, number of cases, and high-risk HPV positivity rate.

Statistical Analysis

Statistical Pack for Social Sciences (SPSS) software version 21.0 (IBM Corp.) was used for all statistical analysis. Continuous variables were given as mean ± standard deviation. Categorical variables were compared by using Fisher-Exact test. A p value less than 0.05 was considered to be statistically significant.

Results

Demographic and Clinical Features

A total of 105 sinonasal carcinomas including 23 incisional biopsies and 82 surgical resection specimens were studied. Among patients, 71 (68%) were men, 34 (32%) were women with a mean age of 56.11 ± 13.93, ranging between 14 and 85. Sixty-four (61%) tumors were identified in the paranasal sinuses (57 [54%] in the maxillary sinus, 3 [3%] in the frontal sinus, 2 [2%] in the ethmoid sinus, 2 [2%] in the sphenoid sinus) and 39 (37%) in the nasal cavity. The most common primary site was the maxillary sinus. In two cases, the primary site could not be identified due to the advanced disease stage.

Pathological Features

Considering the morphological and immunohistochemical features, the cases were classified as follows: keratinizing squamous cell carcinomas (41 [39%]); salivary gland carcinomas (27 [26%]); non-keratinizing squamous cell carcinomas (14 [13%]); sinonasal undifferentiated carcinomas (8 [8%]); adenocarcinomas (7 [7%]); lymphoepithelial carcinomas (3 [3%]); neuroendocrine carcinomas (2 [2%]); adenosquamous carcinoma (1 [1%]); spindle cell squamous cell carcinoma (1 [1%]); verrucous carcinoma (1 [1%]). There were no basaloid squamous cell carcinomas or NUT carcinomas. Nine cases were associated with sinonasal papillomas and detailed clinical and pathological features were given in Table 1.

Results of the Immunohistochemistry and the In Situ Hybridization

P16 immunohistochemistry and HPV DNA ISH results of each tumor type were summarized in Table 2. All control sections were convincingly positive for p16 immunohistochemistry and HPV DNA ISH.

We detected only two HPV-positive cases (Table 3). They showed a typical non-keratinizing morphology showing ribbon-like tumor nests with central necrosis and both were diffusely positive for p16 (Figure 1A-B). In one of these cases, the discrete, dot-like navy-blue nuclear signals (integrative pattern) were readily seen at 40-100x magnifications (Figure 1C), while in the other one, the signals were so weak, which were only detectable

Table 2. P16 Immunohistochemistry and HPV DNA ISH Results of Each Sinonasal Tumor Type.

Histological type	p16 IHC (10%-65%) (%)	p16 IHC (≥70%)* (%)	HPV DNA ISH (%)
Keratinizing SCC	1/41 (2)	2/41 (5)	0/41 (0)
Non-keratinizing SCC	4/14 (29)	3/14 (21)	2/14 (14)
Spindle cell SCC	0/1 (0)	0/1 (0)	0/1 (0)
Verrucous carcinoma	0/1 (0)	0/1 (0)	0/1 (0)
Adenosquamous carcinoma	0/1 (0)	0/1 (0)	0/1 (0)
Salivary gland carcinoma	15/27 (55)	7/27 (26)	0/27 (0)
Adenoid cystic carcinoma	14/20 (70)	5/20 (25)	0/20 (0)
Mucoepidermoid carcinoma	1/3 (33)	0/3 (0)	0/3 (0)
Salivary gland carcinoma, not otherwise specified	0/4 (0)	2/4 (50)	0/4 (0)
Lymphoepithelial carcinoma	0/3 (0)	0/3 (0)	0/3 (0)
Neuroendocrine carcinoma	1/2 (50)	0/2 (0)	0/2 (0)
Sinonasal undifferentiated carcinoma	0/8 (0)	3/8 (37)	0/8 (0)
Adenocarcinoma	5/7 (71)	0/7 (0)	0/7 (0)
Intestinal type	3/5 (60)	0/5 (0)	0/5 (0)
Non-intestinal type	2/2 (100)	0/2 (0)	0/2 (0)
Total	**26/105 (25)**	**15/105 (14)**	**2/105 (2)**

*Diffuse and strong expression in ≥%70 of tumor cells scored as positive.
SCC; squamous cell carcinoma, IHC; immunohistochemistry, ISH; in situ hybridization.

Table 3. Clinicopathologic Features of HPV Positive Cases.

Case	Age	Sex	Localization	Histological type	p16 IHC	Follow-up period	Survival
1	66	Female	Maxilla	Non-keratinizing SCC	Positive	100 months	Alive
2	71	Female	Nasal cavity	Non-keratinizing SCC	Positive	13 months	Died of disease

IHC; immunohistochemistry, SCC; squamous cell carcinoma.

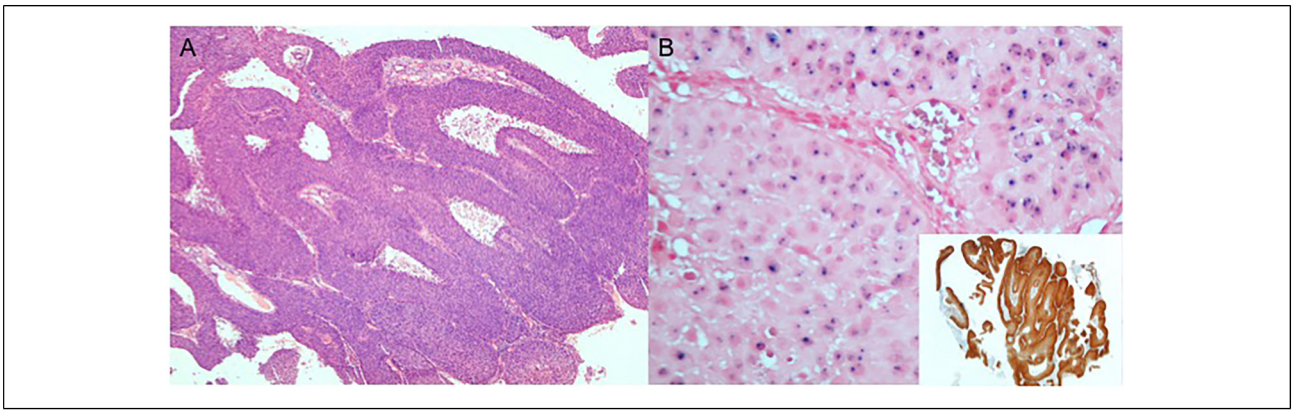


Figure 1. A) HPV-positive cases show prototypic non-keratinizing morphology (H&E, x40) B) blue dot-like positivity for HPV by in situ hybridization (HPV DNA ISH, x400) (inset: diffuse and strong p16 positivity (p16, x20)). (Case 1 in Table 3 is shown).

at 400-1000x magnifications. No staining with the episomal pattern was present in any of these two cases.

In 15 cases, diffuse dust-like blue staining was observed in the background. This finding was referred to a negative result because the signals were both seen in the tumoral and non-tumoral areas, and p16 was negative. Since we recognized that these cases were fixed in Hollande's solution, we thought the background staining might be related to the type of fixation.

In all carcinomas associated with a sinonasal papilloma, both carcinoma and papilloma areas were negative for p16 immunohistochemistry and HPV DNA ISH.

P16 positivity was detected in 15 of 105 cases overall (14%) and was significantly associated with HPV status *($p = 0.02$)*. The sensitivity and the specificity of p16 overexpression in predicting the presence of HPV were 100% and 87%, respectively. However, its positive predictive value was low (2/15, 13%). A total of 13 p16 positive cases were negative for HPV DNA ISH, including 7 salivary gland carcinomas, 3 sinonasal undifferentiated carcinomas, 2 keratinizing squamous cell carcinomas, and 1 non-keratinizing squamous cell carcinoma. Among the p16 negative cases, 64 did not show any expression, while in 26 cases, a varying range of expression between 10% and 60% was observed.

All 20 adenoid cystic carcinomas were negative for HPV DNA ISH. In 19 cases, p16 expression ranged between 30% to 70%, and with a 70% threshold, 5 of them were considered positive. Especially in 10 cases, a biphasic-staining pattern, in which the luminal cells were positive and the abluminal cells were negative, was remarkable (Figure 2).

Prognostic Data

Follow-up information was available for 104 patients (mean follow up $= 44.02 \pm 24$ months, range 1-135). Forty-six (44%) were alive and 58 (56%) died of disease. Since we detected only two HPV positive cases, comparison of overall survival between HPV positive and negative cases could not be performed. One of the two HPV-positive patients died of the disease; the other one was alive at 100 months of follow-up (Table 3).

Systematic Literature Review

A summary of a systematic review of the literature for high-risk HPV incidence among sinonasal carcinomas was given in Table 4.[4–8,11,13–38] As an HPV testing method, only DNA PCR was used in fifteen studies, only DNA ISH was used in two studies, only RNA ISH was used in four studies, at least two methods were used in six studies, and the method was not specified in four studies. Most studies included only squamous cell carcinoma cases, and the HPV rate ranged between 6% and 53%. Three articles studied all types of sinonasal carcinomas and high-risk HPV positivity was 21-25%.[4,15,20]

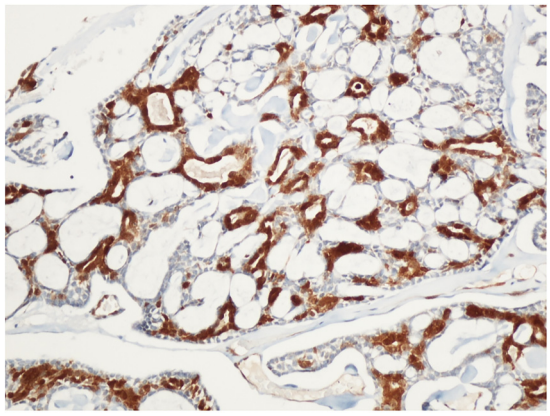


Figure 2. Biphasic p16 expression in adenoid cystic carcinoma (p16, x200).

Discussion

The prevalence of HPV infection shows a distribution specific to the anatomical location in the head and neck, and the oropharynx takes the first place.[1] The sinonasal tract is the second most common site of HPV-related malignancies of the head and neck, and high-risk HPV DNA was found in 21-25% of sinonasal carcinomas.[4,15,20] By histologic subtypes, squamous cell carcinoma is the most commonly associated tumor type with a 31% of high-risk HPV positivity rate.[6,7] Similar to the oropharynx, there is a strong correlation between high-risk HPV positivity and non-keratinizing morphology, and the positivity rate reaches up to 43-50% in this instance.[4,6,19] High-risk HPV can also be detected in other sinonasal tumor types/subtypes, including papillary (80%) and basaloid (50%) squamous cell carcinomas, adenosquamous carcinomas (83%), small cell carcinomas (17%), and sinonasal undifferentiated carcinomas (6%).[4] We found only 2 (2%) high-risk HPV-related non-keratinizing squamous cell carcinomas among 105 sinonasal carcinomas of various histologic types by HPV DNA ISH. HPV DNA positivity rate was 14% in non-keratinizing squamous cell carcinomas, and we did not detect HPV DNA in any other types of sinonasal carcinomas. Even in the non-keratinizing group, the positivity rate was quite low compared to the literature discussed above. On the other hand, our finding is consistent with the relatively low incidence of HPV infection and associated carcinomas in Turkey.[39,40] We believe that our results support various rates of HPV-related carcinomas depending on the geographic and ethnic characteristics.[41]

In the oropharynx, HPV-associated carcinomas are frequently seen in younger patients aged 40 to 60 years.[2] Similarly, the mean age of HPV-positive sinonasal squamous cell carcinomas was significantly lower than the HPV-negative group (58 vs. 64 years).[6] Still, they can occur in a very wide range of ages (33 to 87 years).[4] Strikingly, both of our HPV-positive patients were over 65 years of age, but we could not further comment on the association between HPV and the age at the tumor onset.

HPV-positive squamous cell carcinomas were reported to be more common in the nasal cavity (49%), followed by maxillary (19%), ethmoid (19%), and frontal (18%) sinuses.[6] Because of the low number of HPV positive cases in our series, we could not make any further comments on the site-specific frequency of HPV-positive carcinomas in the sinonasal region.

Diffuse-strong cytoplasmic and nuclear p16 positivity was found to indicate high-risk HPV infection, and we used the 70% threshold based on the literature data.[10] In the oropharynx, p16 overexpression with non-keratinizing morphology is sufficient to diagnose an HPV-related carcinoma.[1,10] For the sinonasal tract, most publications demonstrated a strong and statistically significant correlation between p16 overexpression and HPV positivity. However, its positive predictive value was relatively low.[11] We detected 13 p16 + /HPV- cases. The sinonasal undifferentiated carcinoma was the histologic subtype with the highest number of cases (37%) showing diffuse positivity for p16 without being associated with HPV. Similarly, Wadsworth et al [42] demonstrated that their 5 sinonasal undifferentiated carcinomas showed p16 overexpression and all were negative for HPV DNA. As p16 overexpression has also been detected in HPV-negative cases, strong and diffuse p16 expression cannot be used as a surrogate marker for HPV in sinonasal carcinomas without additional HPV-specific testing.[1,10,11,42] In addition, the lack of p16 overexpression does not exclude the possibility of HPV infection, as rare HPV positive cases were reported to be p16 negative.[4,20]

High-risk HPV-specific testing includes PCR and ISH-based methods. Unlike PCR, ISH also allows observing the distribution of the virus in tumor cells and eliminates the possibility of transmission.[9] Therefore, we chose to use a specific DNA ISH-based method combined with p16 immunohistochemistry to detect high-risk HPV-related sinonasal carcinomas. The expression can only be focal and the assessment of HPV DNA ISH can be challenging with non-specific background staining, and staining artifacts.[43] To avoid false-negative results, we performed HPV DNA ISH on two appropriate paraffin blocks if p16 expression was observed in more than 40% of tumor cells. Except Hollande fixed samples, we did not detect any background staining. Recently, HPV RNA ISH has been shown to be more sensitive and had a better correlation with p16 immunohistochemistry.[9,20,44] The additional RNA ISH testing could increase the number of positivity in our series. However, the difficulties in its availability have limited our use of this method. Although the methodology of HPV testing used in our

Table 4. Systematic Literature Review on HPV Incidence among Sinonasal Carcinomas.

Author-Country-Year	Specific HPV Testing Method	Study cohort	Number of HR-HPV-positive cases and detected subtypes	Histologic type of HPV-positive sinonasal carcinoma
Buchwald et al [14] Denmark-2001	HPV DNA PCR (GP5+/GP6+) (Types 6, 11, 16, 18, 31, 33, 35, and 45)	35 SP-SCCs, 1 SP-adenocarcinoma	3 (8%), HPV type 16 or 18	SCC*
El-Mofty et al [15] USA-2005	HPV DNA PCR (16 HR-HPV, 9 LR-HPV)	39 sinonasal carcinomas (All types)	9 (23%); • 7 HPV type 16 • 1 HPV type 18 • 1 HPV type 45	4 KSCCs 4 NKSCCs 1 SNUC
Hoffmann et al [16] Germany-2006	HPV DNA PCR (HPV6/11 and 16, MY09 and MY11)	20 SCCs	4 (20%), HPV type 16	SCC*
Katori et al [17] Japan-2006-	HPV DNA ISH (Type 6/11, 16/18 and 31/33)	12 SCC 7 SP-SCC	5 (26%); • 2 (SCC) HPV type 16/18 • 3 (SP-SCC) HPV type 16/18	SCC*
Alos et al [5] Spain-2009	HPV DNA PCR (SPF10, 25 HR and LR-HPV types)	60 SCCs	12 (20%); • 11 HPV type 16 • 1 HPV type 35	2 KSCCs 6 NKSCCs 2 basaloid SCCs 2 papillary SCCs
Hasegawa et al [18] Japan-2012	HPV DNA PCR (type 16)	11 SCCs	3 (27%), HPV type 16	SCC*
Bishop et al [4] USA-2013	HPV DNA ISH (HR-cocktail, and types 16, 18, 31/33)	161 sinonasal carcinomas (All types)	34 (21%); • 28 HPV type 16 • 2 HPV type 18 • 4 HPV type 31/33	15 NKSCCs 4 basaloid SCCs 4 papillary SCCs 5 ASCs 1 small cell carcinoma 1 SNUC 4 HMSCs
Larque et al [19] Spain-2014	HPV DNA ISH (HR-cocktail) HPV DNA PCR (SPF10) HPV16 E7 mRNA PCR	70 SCCs	14 (20%); • 13 HPV type 16 • 1 HPV type 35	8 NKSCCs 2 basaloid SCCs 2 papillary SCCs 2 KSCCs
Laco et al [20] The Czech Republic-2015	HPV DNA ISH (HR-cocktail) HPV RNA ISH (HR-cocktail) HPV DNA PCR (GP5+/GP6+) HPV E6/E7 mRNA PCR	73 sinonasal carcinomas (All types)	18 (25%); • 4 HPV type 16 • 5 HPV type 18 • 1 HPV type 35 • 8 HPV other HR	13 NKSCCs 2 KSCCs 2 basaloid SCCs 1 small cell carcinoma
Yamashita et al [21] Japan-China-2015	HPV DNA PCR (GP5+/GP6+)	16 SCCs 5 SP-SCCs	6 (29%); • 3 (SCC) HPV type 16 • 1 (SCC) HPV type 18 • 2 (SP-SCC) HPV type 16	SCC*

(continued)

Table 4. (continued)

Author-Country-Year	Specific HPV Testing Method	Study cohort	Number of HR-HPV-positive cases and detected subtypes	Histologic type of HPV-positive sinonasal carcinoma
Chung et al [22] USA-2015	Next gene sequencing HPV DNA ISH (HR-cocktail)	26 SCCs	14 (54%)	SCC*
Becker et al [23] Germany-2016	HPV DNA PCR (Type 3.5 LCD)	39 SCCs	3 (8%); • 2 HPV type 16 • 1 HPV type 16 and 90	SCC*
Kılıç et al [6] USA-2017	Not available	770 SCCs	244 (32%)	136 SCCs, NOS 37 NKSCCs 37 KSCCs 26 basaloid SCCs 8 papillary SCCs
Chowdhury et al [24] USA-2017	HPV DNA PCR (15 HR types)	26 SCCs	16 (62%); • 8 HPV type 16 • 2 HPV type 18	SCC*
Beigh et al [25] India-2018	HPV DNA PCR (LR and HR)	10 SCCs	2 (20%); • 1 HPV type 16 • 1 HPV type 18	SCC*
Sahnane et al [26] Italy-2018	HPV DNA ISH (HR cocktail and LR cocktail) HPV DNA PCR (HR, for p16+, ISH- cases)	12 SCCs 19 SP-SCCs	3 (10%); • 2 HPV type 16 • 1 HPV other types (31, 33, 35, 52 or 58)	1 SCC* 2 SP-SCCs*
Udager et al [27] USA-2018	HPV DNA PCR (GP5+/GP6+)	14 SCCs 22 SP-SCCs	5 (14%); • 4 (SCC) • 2 HPV type 16 • 1 HPV type 18 • 1 HPV type 33 • 1 (SP-SCC) HPV type 16	4 SCCs* 1 SP-SCCs*
Li et al [8] USA-2018	Not available	581 SCCs	137 (24%)	SCC*
Hsieh et al [28] Taiwan-2018	HPV RNA ISH (HR cocktail) HPV DNA ISH (HR cocktail)	24 non-SCCs	6 (25%)	HMSC
Bulane et al [13] South Africa-2019	HPV DNA PCR (PGMY09/11, and GP5+/GP6+)	25 SCCs	4 (16%); • 1 HPV type 16 • 1 HPV type 18 • 2 HPV type 45	SCC*
Jiromaru et al [11] Japan-2020	HPV RNA ISH (HR cocktail)	101 SCCs	9 (9%)	SCC*

(continued)

Table 4. (continued)

Author-Country-Year	Specific HPV Testing Method	Study cohort	Number of HR-HPV-positive cases and detected subtypes	Histologic type of HPV-positive sinonasal carcinoma
Oliver et al [7] USA-2020	Not available	1418 SCCs	447 (32%)	SCC*
Hu et al [29] China-2020	HPV DNA PCR	30 SP-SCCs 84 SCCs	8 (7%); • 6 (SP-SCC) HPV type 16 • 2 (SCC) HPV type 16 • 2 HPV type 16	6 SP-SCC* 2 SCC*
Cohen et al [30] USA-2020	HPV RNA ISH (type 16, 18, 31, 33)	22 SCCs		SCC*
Cabal et al [31] Spain-2020	HPV DNA PCR (MY11/GP6+)	13 SP-SCCs 50 SCCs	6 (9%) • 5 (SP-SCC) • 2 HPV type 16 • 1 HPV type 18 • 1 HPV type 42/61 • 1 unknown 1 (SCC) HPV type 16	5 SP-SCCs* 1 SCC*
Kim et al [32] Korea-2020	HPV DNA PCR (14 HR+5 LR types)	14 SCCs	3 (21%); • 2 HPV type 16 • 1 HPV type 69	SCC*
Al-Qurayshi et al [33] USA-2020	Not available	96 SCCs	24 (25%)	SCC*
Svajdler et al [34] The Czech Republic-Slovakia-2020	HR-HPV DNA PCR (CPSGB, GP5+/GP6+, and type-specific primers for HPV 16, 18, 31, 33, 35, 45) HPV16 E6 mRNA PCR HPV RNA ISH (HR-cocktail)	34 SCCs	8 (24%); • 5 HPV type 16 • 1 HPV type 18 • 1 HPV type 45 • 1 HPV other types	SCC*
Markovic et al [35] USA-2020	HPV DNA PCR (HPV16/18, GP5+/GP6+)	32 SCCs	17 (53%); • 6 HPV type 16 • 2 HPV type 18 • 2 HPV type 33 • 1 HPV type 35 • 1 HPV type 45 • 1 HPV type 56 • 1 HPV type 69 • 1 HPV type 11, 16 • 1 HPV type 16, 33 • 1 HPV type 16,18, 45	6 KSCCs 7 NKSCCs 4 papillary SCCs 3 basaloid SCCs

(continued)

Table 4. (continued)

Author-Country-Year	Specific HPV Testing Method	Study cohort	Number of HR-HPV-positive cases and detected subtypes	Histologic type of HPV-positive sinonasal carcinoma
Hongo et al [36] Japan-2021	HPV RNA ISH (HR-cocktail)	131 SCCs	8 (6%)	SCC*
Nishikawa et al [37] Japan-2021	HPV DNA PCR (GP5+/GP6+)	85 SCCs	7 (8%); • 1 HPV type 16 • 3 HPV type 18 • 3 HPV type 33	SCC*
Hongo et al [38] Japan-2021	HPV RNA ISH (HR-cocktail)	14 SP-SCCs 132 SCCs	11 (8%) (de novo SCC)	SCC*

*Not otherwise classified.

Abbreviations: ASC, adenosquamous carcinoma; HMSC, HPV-related multiphenotypic sinonasal carcinoma; HR-HPV, high-risk human papilloma virus; ISH, in situ hybridization; KSCC, keratinizing squamous cell carcinoma; NKSCC, non-keratinizing squamous cell carcinoma; SCC, squamous cell carcinoma; SNUC, sinonasal undifferentiated carcinoma; SP, sinonasal papilloma.

study is suboptimal, the frequency of HPV-positive sinonasal carcinomas we detected is compatible with the previous data on HPV incidence from Turkey.[39,40]

HPV-related head and neck carcinomas prototypically show a non-keratinizing squamous morphology.[1] In recent years, Bishop et al [45] described a distinctive variant of sinonasal carcinoma, now termed HPV-related multiphenotypic sinonasal carcinoma. Those tumors morphologically resemble salivary gland carcinomas, especially solid variant of adenoid cystic carcinomas, with their solid and cribriform patterns. The glandular structures are composed of dual cell population and basaloid cells display myoepithelial phenotype. The presence of squamous dysplasia in the surface epithelium, detection of high-risk HPV, particularly type 33, and absence of *MYB, MYBL1,* or *NFIB* gene fusions may be helpful to distinguish those tumors from salivary gland carcinomas.[45,46] In our series, all salivary gland tumors including 20 adenoid cystic carcinomas were negative for HPV by ISH. Similar to Laco et al ,[20] we also observed a biphasic-staining pattern for p16 in adenoid cystic carcinomas, in which luminal cells were positive and abluminal cells were remarkably negative. In five cases, p16 expression reached the 70% threshold and was scored as positive. The nonspecific overexpression of p16 regardless of HPV status makes p16 immunohistochemistry difficult to use as a reliable surrogate marker in multiphenotypic sinonasal carcinomas. High-risk HPV detection assays can be used to diagnose this rare subtype of HPV-related sinonasal carcinomas.

Sinonasal papillomas are rare benign neoplasms of the sinonasal tract, and 5% to 27% of them may undergo malignant transformation into squamous cell carcinoma.[47] We here reported nine inverted papillomas with associated malignancy with one verrucous carcinoma, which is extremely rare in the literature.[48,49] The mechanism of malignant transformation has not yet been well-understood. Udager et al [50] found identical *EGFR* mutations in both tumor components that may suggest the common origin of a papilloma and an associated carcinoma. The role of HPV infection as a driver in malignant transformation is also debated. Recently, Mehrad et al [51] found that a small subset of inverted papillomas is associated with transcriptionally active low-risk HPV infection by using RNA-based HPV detection methods. Although that suggests a possible increase in the risk of malignancy, association with malignant transformation remains unclear. We searched high-risk HPV infection by using HPV DNA ISH and p16 immunohistochemistry and all inverted papillomas and associated carcinomas were negative. Similarly, Rooper et al [52] did not detect transcriptionally active high-risk HPV by HPV RNA ISH in any of their 16 inverted papillomas with carcinomatous transformation. According to these results, high-risk HPV does not appear to have an important role in the development of malignant transformation.

HPV-related carcinomas are associated with improved outcomes and are more responsive to radiotherapy and chemotherapy in the oropharynx.[2,3] Whether HPV-positive sinonasal squamous cell carcinomas have the same prognostic advantage is controversial.[4–8] Li et al [8] studied 581 sinonasal carcinomas and found that HPV positivity is not an important prognostic factor in the sinonasal tract. On the contrary, Oliver et al concluded that HPV positivity is associated with improved overall survival, and suggest routine HPV testing in sinonasal squamous cell carcinomas. We could not make further analysis on prognosis due to the limited number of HPV-positive cases in our study.

Our thorough literature review on high-risk HPV in the sinonasal tract demonstrated differences among series for many reasons, such as the nations, the type of the tumor included in the study, the method used, and the HPV subtype studied (Table 4). Among the articles that included only squamous cell carcinomas, the lowest positivity rate was from Japan with 6%, and the highest rate was from the USA with 53%.[35,36] Our positivity rate was just 4% considering only squamous cell carcinomas. On the other hand, HPV infection and associated carcinoma rates are low in Turkey.[39,40] According to the national cancer registry data of the Turkish Ministry of Health, cervical cancer incidence is extremely low when compared with European and Asian countries.[39] There are limited publications on the association between head and neck cancers and high-risk HPV, focusing on oropharyngeal and laryngeal carcinomas. HPV positivity rate has been reported as 52% in oropharyngeal carcinomas and 12-26% in laryngeal carcinomas, slightly lower than in Western countries.[53–55] However, during the past decade, HPV positivity has been increasing in Turkish patients as worldwide.[54,55] As far as we know, our study is the first study from Turkey on high-risk HPV and sinonasal carcinomas. We found that the positivity rate was quite low compared to the literature, and this finding needs to be supported by further studies.

There are some limitations of this study. We searched high-risk HPV using only one HPV-specific testing method (HPV DNA ISH) combined with p16 immunohistochemistry. The sensitivity of HPV DNA ISH is relatively low due to focal expression, and the assessment can be challenging with background non-specific staining and staining artifacts.[43,44] Although performing additional HPV specific testing may increase the sensitivity and the number of positive cases, we think that it may not make a significant difference considering the low percentage of p16 positivity in our cases and low incidence of HPV in our geographic region. Furthermore, our small number of HPV-positive cases precluded us from comparing

clinicopathological features between HPV-positive and HPV-negative carcinomas.

In conclusion, our series demonstrates a much lower percentage of HPV positivity in sinonasal carcinomas than previously reported in the literature. In accordance with the low incidence of HPV infection and associated neoplasms in Turkey, HPV seems to have a limited role as an etiologic agent in sinonasal tumors.[39,40] We believe that our study is a peculiar example of the importance of the geographic distribution of HPV infection, and HPV prevalence should also be taken into consideration in the decision of HPV screening programs. Moreover, the positive predictive value of p16 positivity to detect high-risk HPV-related sinonasal carcinomas is relatively low and should be interpreted carefully along with the morphological features in the sinonasal tract.[11,42]

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Statement of Ethics: This retrospective study was approved by the institutional ethics committee of Istanbul University, Istanbul Faculty of Medicine (Issue number: 2017/67).

Author Contributions

LA and MU contributed to the acquisition of clinical data, surgical treatments, patient follow-ups, and manuscript review; DY participated in study design and manuscript review; EAA and YO contributed to the study design, analysis, and interpretation of data, manuscript preparation, and revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Ethical Approval

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Trial Registration

Not applicable, because this article does not contain any clinical trials.

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