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Original Article

Exploring the Role of HPV 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx

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ABSTRACT

Objective: Human papillomavirus infections may have a role in the development of oral cavity and oropharynx carcinomas. Human papillomavirus-positive oral cavity and oropharyngeal carcinomas differ from human papillomavirus-negative in that to occur in younger, are more frequent in men, and are strongly associated with sexual behavior. These observations lead to the treatment options and outcomes in human papillomavirus-related tumors, and the questions of targeted treatment that can be performed in the coming years have come to age.

Methods: This prospective study was conducted at Gaziantep University, medical faculty, otorhinolaryngology department. Patients with squamous cell carcinomas of non-lip oral cavity and oropharyngeal admitted to our department were included in the study. Samples from the cases were immunohistochemically stained. Sections were examined by light microscopy.

Results: The 55 cases P16 (76.4%) expressions were detected to be positive, and 17 (23.6%) cases were negative. There was no statistically significant correlation between prognostic parameters and p16 expressions. However, a significant difference was detected between human papillomavirus-positive and negative groups in regard to survival in oropharyngeal carcinoma.

Conclusion: Disease management can consider human papillomavirus-positive oral cavity and oropharyngeal carcinomas as a separate group. human papillomavirus-positive oral cavity and oropharyngeal carcinomas respond better to chemotherapy and radiotherapy than human papilloma virus-negative cancers. The presence/absence of human papillomavirus 16 might be considered a prognostic marker, but its reliability has not yet been confirmed. In future clinical studies, cancer centers should classify head–neck patients with respect to human papillomavirus status. However, we must always emphasize that the best treatment for cancer in which the main pathogenic agent is known is protection.

Keywords: Oral cavity, oropharynx, neoplasms, human papillomavirus p16, immunohistochemistry

INTRODUCTION

Head and neck carcinoma is the sixth most common cancer in the whole world.[1] Head–neck cancers are more common in males and occur in fifth and sixth decades.[2] Ninety percent of cancer that appeared in head–neck region are squamous cell carcinomas (SCC). Oral cavity and oropharynx cancers are the most common cancers all over the world and the second most common cancer in our country.

The relationship between smoking and alcohol and the cancers of the oral cavity and oropharynx has been known for a long time. The opinions on some factors such as diet and oral hygiene predispose to the disease have been expressed.[3,4] Animal studies have been performed to light on the relationship between head–neck cancer and hereditary, which has begun to focus on human papillomavirus (HPV) infections in addition to other factors in recent years.[3-5] It has been understood that DNA viruses can create tumors in mammals, and Shope[6] has shown keratinous lesions to be formed in rabbits following papillomavirus infections in 1993, and some of them have also transformed into epithelial neoplasms.

Human papillomavirus is a DNA group virus in the family of Papovaviridea in which 200 different types have been identified. Molecular studies indicate that specific mechanisms play a role in HPV-induced carcinogenesis, and it has been thought to have a relationship between HPV infection and head and neck cancers.[5,7]

Various studies have shown that some specific HPV types are associated with many premalignant and malignant lesions of the cervix uteri, vulva, penis, conjunctiva, and upper respirator

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y–digestive system.[8] These patients' carcinoembryonic antigen levels increased, and such cellular immunosuppression may predispose to cancer. Methods used in virus detection are election microscope, immunohistochemical staining, hybridization techniques (Southern blot, dot blot, and in situ hybridization), and "polymerase chain reaction" (PCR). This causal association between HPV and SCCs suggests that the presence of the virus may be a high-risk indicator between HPV and SCCs. Brandwein et al[9] reported that the presence of HPV DNA in laryngeal tumors was associated with prognosis.

The present study aimed to investigate HPV p16 presence in the oral cavity and oropharynx carcinomas using histochemical methods. The expected benefits of this study are to demonstrate the relationship of HPV p16 with clinicopathologic parameters in oral cavity and oropharyngeal carcinomas, to determine the behavior model of oral cavity and oropharynx cancers in advance, and to provide the most appropriate methods for treatment.

METHODS

This study aimed to indicate the effects of the relationships of HPV 16 with oral cavity and oropharynx cancers on age, stage, relapse, metastasis, and 3-year survival. The patients were examined retrospectively. This study was approved by Gaziantep University Clinical Research Ethics Committee (Date: April 6, 2015, 2015/114).

The patients with oral cavity and oropharynx SCCs and admitted to polyclinic of Department of Otorhinolaryngology of Gaziantep University Faculty of Medicine in 2002-2015 were included in this study. The patients with lip carcinoma, histopathology other than SCC, previously treated and with additional malignancies were excluded from the study.

After receiving the detailed history of the patient who meets the above characteristics, a head and neck examination was performed and a histopathologic diagnosis was made by biopsy. Following histopathological diagnosis, at least one of the treatment methods of excision, neck dissection with excision, or chemotherapy/radiotherapy was applied to the patients. Patients with squamous epithelial cell carcinoma of language, hard palate, buccal mucosa, retromolar triangle, soft palate, tonsil, and tongue were included in our study. The case data are collected as follows:

Main Points

- Oral cavity and oropharynx squamous cell cancers should be examined for human papillomavirus (HPV) 16 positivity.
- Immunohistochemical examination is a suitable method for the diagnosis of HPV 16.
- HPV 16 positivity can be evaluated as a prognostic factor in oral cavity and oropharynx squamous cell cancers.
- Prophylactic vaccination studies should be carried out to prevent cancers that are known to be the main pathogenic agent such as HPV 16.

- General information about the demographic, medical, and current illnesses of the cases was taken from personal information form and pathology records that were routinely filled at the center where the study was conducted.
- The success of the surgeon after surgery and metastasis and relapse developments was followed by the file records and pathology records of the cases.

Immunohistochemically Staining

The study consisted of 72 cases who operated due to non-lip oral cavity and oropharynx squamous cell cancer at Otorhinolaryngology Gaziantep University Faculty of Medicine and whose specimens were sent to the Laboratory of Pathology in 2002-2015.

Paraffin blocks were sectioned with a "Leica RM 2145" model microtome to a thickness of 4 μm and followed pre-staining protocols. Subsequently, p16 antibody was immunohistochemically administrated using CINtec Histology kit containing E6H4 clone antibody against P16INK4a. Nuclear and cytoplasmic staining is the basis. Staining of over 70% was considered positive. Figure 1a and b shows the positive p16 light microscope image of SCC (×100) and Figure 2a and b shows the image of a p16 negative patient with the same disease.

Statistical Analysis

Statistical Package for the Social Sciences 22.0 (IBM Corporation, Armonk, NY, USA) program was used to analyze the variables. Normal distribution suitability of univariate variables was assessed by Lilliefors corrected Kolmogorov–Smirnov test and variance homogeneity was assessed by the Levene test. Independent–Samples *t* test was used together with Bootstrap results for comparing 2 independent groups. When comparing categorical variables, Pearson chi- and Fisher's exact tests were tested with Monte Carlo Simulation technique. The odds ratio was used to determine the most important risk factor among categorical significant risk factors. The Kaplan–Meier (product-limit method)-LogRank (Mantel-Cox) analysis was used to examine the effect of factors on mortality and lifespan. Quantitative variables were tabulated to be ±std. (standard deviation) and range (maximum–minimum), and categorical variables were shown as n (%). Variables were examined at 95% confidence level and $P < .05$ was considered significant.

RESULTS

This study included 72 patients diagnosed with non-lip oral cavity and oropharyngeal SCC at the Department of Otorhinolaryngology Gaziantep University Faculty of Medicine. Totally 26 patients (36.1%) were female, and 46 patients (63.9%) were male. The age distribution ranged from 16 to 88 (mean, 53.39). We examined the patients after dividing them first into 2 groups as oral cavity and oropharyngeal carcinomas and then grouped as positive and negative according to HPV p16 staining. The disease was located in the oropharynx of 20 patients (27.7%) and in the oral cavity of 52 patients (72.3%). Distribution of the disease in cases according to localization is shown in Figure 3.

Figure 1. A, B. Histopathological view of squamous cell carcinoma (H–E, ×100) (a) and positive p 16 image of same patient (×100) (b). H–E, hematoxylin and eosin.

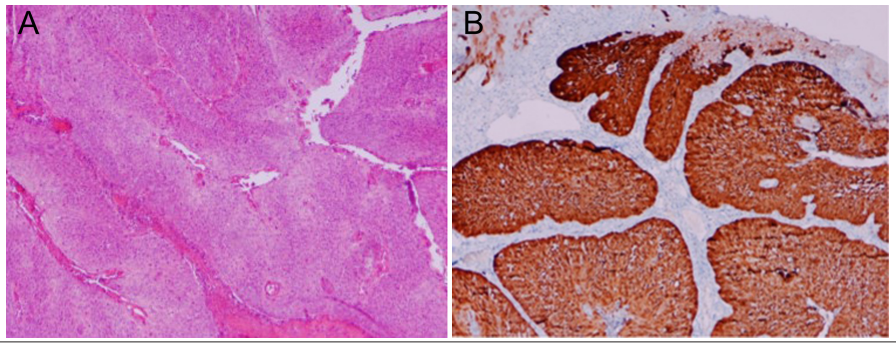


Figure 2. A, B. Histopathological view of squamous cell carcinoma (H–E, ×100) (a) and negative p 16 image of same patient (×100) (b). H–E, hematoxylin and eosin.

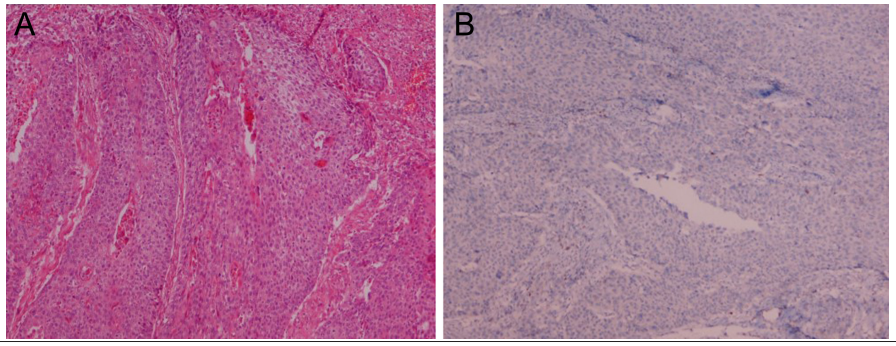
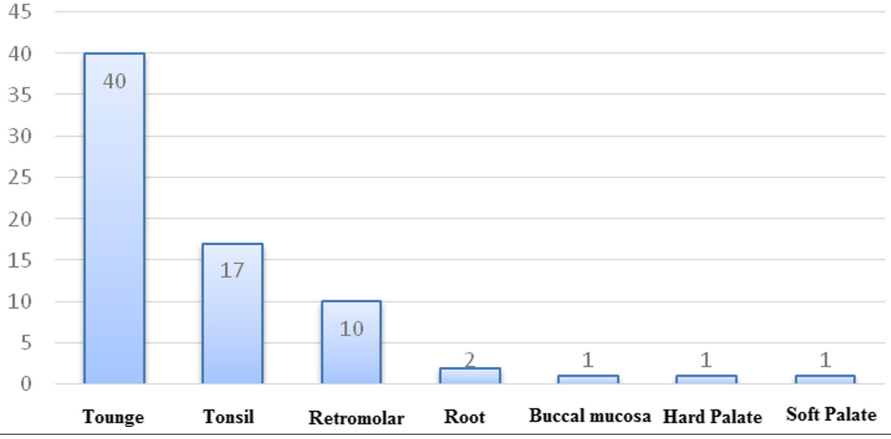


Figure 3. Distribution of cases based on localization.

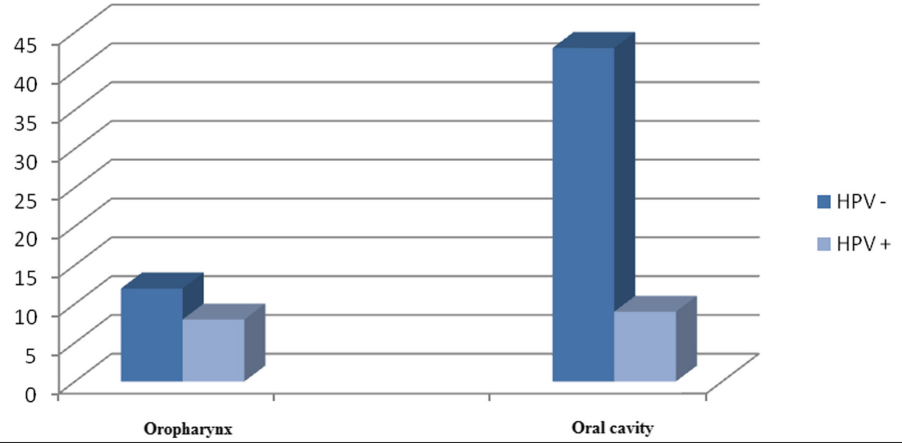


Totally 17 (85%) cases with oropharyngeal cancers were male and 3 (15%) were female and 29 (55.8%) of the patients with oral cavity cancer were male and 23 (44.2%) were female. Oral cavity and oropharynx incidence were found statistically higher in males than females ($P = .028$)

Human papillomavirus p16 was found to be positive in 17 of 72 (23.6%) patients included in the study. Nine of 52 cases (17.3%) with oral cavity cancer were found to have HPV p16 positivity and 8 of 20 cases (40%) with oropharynx cancer were found to have HPV p16 positivity. A statistical difference was not recorded between oral cavity and oropharynx cancer in terms of HPV positivity ($P = .063$; Figure 4).

A total of 63 (87.5%) patients were operated on, 9 (12.5%) patients underwent chemotherapy and radiotherapy after

Figure 4. Distribution of oral cavity and oropharynx cancers based on HPV p16 staining. HPV, human papillomavirus.



biopsy and histopathological diagnosis of SCC, and 62 surgical excisions and neck dissections were simultaneously performed in 62 of the patients who were operated, and only 1 patient was surgically excised.

When 63 patients were evaluated in terms of stage, 15 patients (23.8%) were stage 1, 14 patients (22.2%) were stage 2, 13 patients (20.6%) were stage 3, and 21 patients (33.3%) were stage 4 (Figure 5).

While there was not a significant difference in terms of stage between HPV negative and positive groups in oropharynx cancers ($P = .424$), there was a significant difference in terms of stage between HPV positive and negative groups in oral cavity cancers ($P = .017$). Human papillomavirus-positive group in oral cavitary cancers was seen at an earlier stage (Table 1).

We performed follow-up visits for our operated patients with physical examination and imaging methods in our clinic, of which we observed metastasis to the neck lymph nodes in 26 of (41.2%) 63 patients operated and relapse in 20 (31.7%). A significant difference between HPV-positive and -negative groups in oral cavity and oropharynx cancers in terms of metastasis to neck lymph nodes was not recorded. (oral cavity $P = .240$, oropharynx $P = 1$) (Table 2).

However, 3-year survival rate of HPV-positive group was statistically higher than HPV-negative group ($P = .032$).

When the difference between sex and 3-year survival rate is evaluated, although no significant difference was found between male and female groups in terms of 3-year survival in oral cavity cancers ($P = .381$), a significant difference was found between male and female groups in terms of 3-year survival in oropharynx cancers ($P = .001$). Three-year survival in oropharynx cancers was found to be significantly worse in women.

When 3-year survival is evaluated between the patients operated on, and the patients who underwent chemotherapy/radiotherapy in oral cavity and oropharynx, a significant

Figure 5. Distribution of oral cavity and oropharynx cancer in terms of stage.

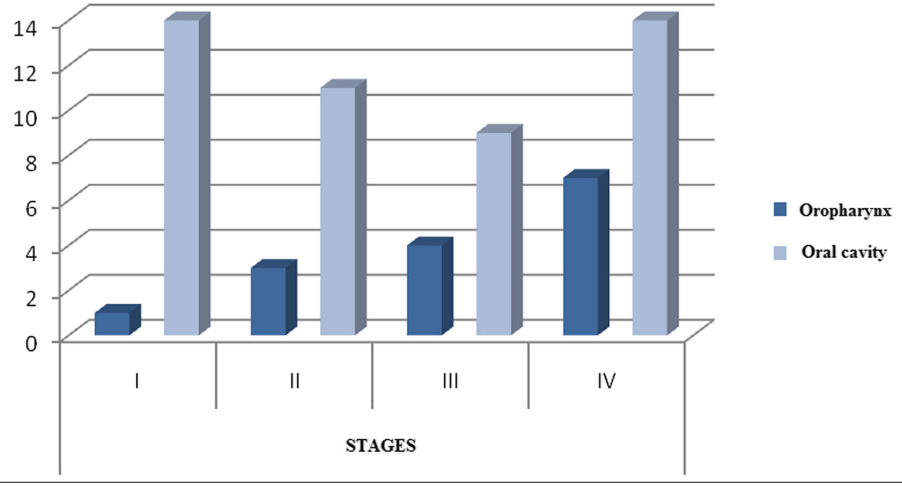


Table 1. Comparison of the Relationship Between HPV and Stages

		STAGES					
		I	II	III	IV		
	HPV	n (%)	n (%)	n (%)	n (%)	Total	*P*
Oropharynx	–	1 (14.3)	2 (28.6)	2 (28.6)	2 (28.6)	7 (100)	.424
	+	0 (0.0)	1 (12.5)	2 (25.0)	5 (62.5)	8 (100)	
Oral cavity	–	12 (30.0)	6 (15.0)	8 (20.0)	14 (35.0)	40 (100)	.017
	+	2 (25.0)	5 (62.5)	1 (12.5)	0 (0.0)	8 (100)	
Total	–	13 (27.7)	8 (17.0)	10 (21.3)	16 (34.0)	47 (100)	.330
	+	2 (12.5)	6 (37.5)	3 (18.8)	5 (31.3)	16 (100)	

Pearson chi–square test (Monte Carlo).
HPV, human papillomavirus.

Table 2. Comparison of the Relationship Between HPV and Metastasis

		Localization					
		Oropharynx		Oral Cavity		Total	
		HPV		HPV		HPV	
		–	+	–	+	–	+
Metastasis	None	3 (42.9)	2 (25.0)	25 (62.5)	7 (87.5)	28 (59.6)	9 (56.3)
	Positive	4 (57.1)	6 (75.0)	15 (37.5)	1 (12.5)	19 (40.4)	7 (43.8)
P		1		0.240		1	
Relapse	None	5 (71.4)	6 (75.0)	27 (67.5)	5 (62.5)	32 (68.1)	11 (68.8)
	Positive	2 (28.6)	2 (25.0)	13 (32.5)	3 (37.5)	15 (31.9)	5 (31.3)
P		1		1		1	

Fisher's exact test (Exact).
HPV, human papillomavirus.

difference was recorded between operated patients and those who underwent chemotherapy/radiotherapy in both oral cavity and oropharynx cancer groups (oral cavity cancers $P = .001$, oropharynx cancers $P = .016$). Three-year survival rate of operated patients was found statistically better in oral and oropharyngeal cancers.

When the relationship between the presence of neck lymph node metastasis and 3-year survival is evaluated, no significant difference in the 3-year survival rate between patients with or without metastases in oropharyngeal carcinomas was observed ($P = .611$); however, a significant difference was found between patients with or without metastasis in oral cavity cancer ($P = .049$), and non-metastatic group's 3-year survival rate was found statistically higher than metastatic group.

When the relation between relapse and 3-year survival is evaluated, no significant difference was found between the groups with or without relapse in oral cavity cancers ($P = .115$), however, a significant difference between the groups with or without relapse was found in oropharynx cancers ($P = .046$), in which non-relapsing group was higher.

DISCUSSION

The incidence of head and neck region cancers was found to be less than 5% of all cancers in developed countries. This percentage reaches up to 17 in developing countries. Oral cavity cancers constitute 25%-35% of head and neck cancers and occur 3 times more in men than in women between the age of 50 and 60.[10] These cancers are one of the major health problems with increasing frequency in many parts of the world. Despite recent advances in treatment and new protocols using alternative treatment modalities, the prognosis of patients is still poor. When lesion and treatments-caused functional and cosmetic deformities are combined with low survival rate (5-year survival rate T1-T2: 51%, T3-T4: 18%,[11] the importance of oral and oropharynx cancers is increasing even more. Although there are improvements in CT, RT, and surgical treatment techniques, the survival rates of patients have increased very little in recent years, which makes it necessary to investigate the treatment methods causing

the least mortality and morbidity. The most important factor for effective treatment is early diagnosis, which allows aesthetic, functional, and oncological successful outcomes.[11]

In general, it is thought that the most reliable parameters in treatment planning and prognostic determination can be determined by tumor node metastasis (TNM) classification. Lymph node metastasis is the most important parameter accepted.

However, even if all these features are taken into account and the same treatment modalities are administrated to the patients, there can be significant differences in terms of treatment response, relapse, tumor behavior, and overall prognosis among the patients. These differences lead to the conclusion that there are other factors affecting the outcome of oral cavity and oropharynx cancer treatment, and recently, some researchers thought viral factors might be the reason for differences.

Many studies have shown that smoking and alcohol use are major, common risk factors for head and neck SCC (HNSCC). However, for the last 10-15 years, HPV infection has been recognized as a major etiologic risk factor for a type of HNSCC,[12,13] which is mostly oropharyngeal SCC (OPSCC). For the first time, Gillison et al[14] reported that HPV infection plays a role in OPSCC etiology. Many case studies have been conducted to evaluate the prevalence of HPV infection in oropharyngeal cancers using molecular techniques such as PCR or in situ hybridization in 2000.[15,16] In fact, it has been very clear for the last 5 years that HPV plays a pathogenic role in head and neck cancers. These findings provide new opportunities for advanced therapy and primary prevention for HNSCC.[17]

It has been known for almost a century that HPV is in a relation to upper respiratory tract pathologies. However, the viral oncogenic effects have been better reported in the literature in the last 3 decades.[18-20] Human papilloma virus has been found to be associated with oropharyngeal cancers, especially tonsil cancers. The life span of HPV-positive cases and the therapeutic response were thought to be better than HPV-negative cases.[21,22]

Human papillomavirus is a DNA virus with more than 200 types defined in the PapovaViridea family.

Human papillomavirus prevents apoptosis in human genital keratinocytes and oral and tonsillar epithelial cells. Tissue culture derived from immortalized cell line results in a transformed phenotype. This data indicate that HPV plays initiator role in the transformation of malignant.

Immunohistochemical staining, hybridization techniques (Southern blot, dot blot, and in situ hybridization), and PCR techniques are used to detect viruses. But, which one of these techniques is safety is still being discussed.[23,24]

This causal relationship between HPV and SCCs suggests that the presence of the virus may be a high risk of developing cancer. The high-risk subtypes of HPV are HPV 16, 18, 31, 33, 39, 45, 52, 58, and 69 and play role in cervical and other anogenital cancers. Human papillomavirus 6 and 11 are "low risk" types and are rarely seen in malign lesions. They mainly occur in non-malignant lesions.

In some studies, the reasons for HPV infections in head and neck regions are reported as oral–genital contact, multiple sex partners, infection from mother to baby during childbirth, and hygenic behavior differences.[25]

D'Souza et al[26] reported in a case–control study that the high number of vaginal sex partners (>26) and 6 or more oral sex partners are high-risk factor for OPSCC. In women with HPV-induced anogenital cancer, the risk of HPV-induced OPSCC risk is also increased. Also, male partners of these patients had HPV contamination in oropharyngeal cavities have been seen, which has been supported by the studies of Frisch[27] and Hemminki.[28]

There are many great studies in literature that investigated HPV prevalence in head and neck cancers, which has been detected at 34.5%. However, a wide range of about 7%-59% has been found, depending on the localization of the selected tumor group, the method used, or the patient characteristics. In our study, 17 of all cases (23.6%) have been detected to be positive by immunohistochemical staining method. In our study, although HPV is positive in 40% of oropharynx cancers, it is positive in 17.3% of oral cavity cancers. This ratio is statistically significant, but HPV positivity was found to be high (40 %) in oropharynx cancers, and we have concluded that small number of cases lead this ratio to be statistically insignificant results.

Miller et al[29] found HPV to be in the ratios of 10% in normal oral mucosa, 22.2% in leukoplakia, 26.2% in intraepithelial neoplasia, 29.9% in Verrucous carcinoma, and 46.5% oral SCC.

SahebJamee et al[30] investigated the presence of HPV in the saliva of cases with oral SCC and control group using PCR method. Human papillomavirus was found to be positive in 40.9% of SCC cases and 25% of control group. Human papillomavirus 16 was found to be in 27.3% of the cases and 20% of the control group. In this study, the difference between HPV rates in the patient group and the control group was not statistically significant.

Marur et al[4] found that HPV-positive head and neck tumors were more common in males. In the same study, HPV-positive head and neck SCCs were found to be more sensitive to chemotherapy and radiotherapy. They also noted that HPV p16 has an effect on survival but was not sufficient by itself. In our study, HPV-positive tumors were more common in male, especially in oropharynx cancers, and the difference between males and females was statistically significant.

Ang et al[31] showed that HPV-positive patients were generally younger, diagnosed at 54 years of age, and had fewer cigarette and alcohol exposures. In our study, no statistically significant relationship between HPV and age has been recorded.

Many studies have shown that HPV-positive tumors are generally being presented as early T stage (T1, T2)[32] and high N stage

(generally cystic and multilevel)[33] and have generally different histologic features (moderate/weak tumor differentiation and non-keratinization or basaloid pathology).[32,33] In our study, 82.3% of HPV-positive cancers were seen in the early T-phase (T1, T2) and 17.7% in the late T-phase (T3). In terms of neck lymph node metastasis, 68.75% of HPV-positive cancers were seen in early N (N0, N1) and 31.25% in late N (N2, N3). Also, in operated patient group, the distribution of cases is as follows: 15 cases (23. 8%) are in stage 1, 14 cases are in stage 2 (22.2%), 13 cases are in stage 3 (20.6%), and 21 cases are in stage 4 (33.3%). Lymph node metastases were detected in 26 patients (41.2%). In our study, no statistically significant difference was found between HPV-positive group and HPV-negative group in terms of metastasis and recurrence.

In our study, no significant difference between HPV and stages in oropharyngeal carcinomas has been recorded. However, there was a significant difference between HPV and stages in oral cavity cancers. In our study, HPV-positive group in oral cavity cancers was seen especially in stages 1 and 2.

Lim et al[34] have not recorded any significant difference in survival between HPV-positive and -negative groups. Ang et al[31] have shown HPV-positive group to have better prognosis than HPV-negative group. In the same study, HPV positivity was found to have a positive effect on survival. Similar results were obtained in the study of Chaturvedi.[35] In our study, it was found that the survival rate of HPV-positive group was statistically significantly better than HPV-negative group in oropharyngeal carcinoma, while there was no significant difference between HPV-positive group and negative group in oral cavity cancer in terms of 3-year survival.

Studies have shown that HPV is associated with head and neck cancers, especially oropharyngeal cancers. In our study, HPV 16 positivity was found as high as 40% in oropharyngeal carcinomas, but this ratio was not found statistically significant. Studies have shown that HPV-associated cancers occurred in younger age groups. But the age distribution in our study is heterogeneous.

The best viral detection method chosen for tumors is still controversial, and both in situ hybridization and PCR are often used. P16 immunohistochemistry is also used to detect HPV infection. Thus, a new marker is required to define the best treatment option for HPV infection. Besides, the presence/absence of HPV infection can be considered as prognostic marker, but its use has not yet been approved. There are still many questions about oral HPV infection.

In the literature, it is seen that the prognosis of HPV-positive cancers is better, and the survival rate is higher. In our study, it was seen that the 3-year survival rate of oropharynx cancer was higher. It was also observed that oral cavity cancers were at earlier stage.

Human papillomavirus-positive cancers' T stage is consistent with the literature but differs from the literature on early T stage in terms of N stage. In the literature, HPV-positive cancers were seen in late N stage, whereas it is in early N stage in our study.

Human papillomavirus-positive oral cavity and oropharyngeal cancers respond better to chemotherapy and radiotherapy than HPV negative.

The limited numerical data and the fact that only HPV P16 markers were examined were accepted as a limitation of our study. A detailed investigation of the relationship between HPV and oral cavity–oropharynx cancers will provide important contributions to the literature.

CONCLUSION

Regarding disease management, we can consider HPV-positive oral cavity and oropharynx cancers as a separate subgroup of HNSCC because of their more positive results. Human papillomavirus-positive oral cavity and oropharyngeal carcinoma patients are typically younger and have a better general health status. In future clinical trials, cancer centers should classify head and neck patients according to HPV status. Regardless of treatment modality, we have an opportunity to investigate treatment strategies that increase survival rates and reduce the rate of lethal side effects. In other words, our general purpose should be to provide high level of life quality and minimal treatment complications. In some studies, this type of treatment strategy seems to be possible for HPV-induced cancers so new studies to be done in this field are required.

We must always emphasize that the best treatment for cancer, especially the main pathogenic agent, is prevention. The importance of vaccination, especially in HPV-related cancers, has been shown in recent years, so we must emphasize the importance of increasing the number of detailed studies that indicate the impact of vaccination on head and neck cancers.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Gaziantep University (Date: April 6, 2015, Decision no: 2015/114).

Informed Consent: Written and signed informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – K.T., F.Ç., M.K.; Design – K.T., F.Ç., M.K.; Supervision – F.Ç., M.K.; Resources – K.T., E.K.; Materials – K.T., E.K., M.K.; Data Collection and/or Processing – K.T., İ.A., E.K.; Analysis and/or Interpretation – K.T., F.Ç., İ.A., M.K.; Literature Search – K.T., F.Ç., İ.A., E.K.; Writing Manuscript – K.T., İ.A., E.K.; Critical Review – E.A.; Other – F.Ç., İ.A., M.K.

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