Original Research

DOI: 10.5152/EurJTher.2021.21042

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

Authors: Koray Tümüklü[1-0000-0001-8821-4457], Fatih Çelenk[2-0000-0002-7557-7854], İsmail Aytaç[3-0000-0002-0947-366X], Ercan Kurt[4-0000-0003-2595-0873], Muzaffer Kanlikama[3-0000-0002-3537-7778]

Institutions:

[1]Department of Otorhinolaryngology, Sanko University Faculty of Medicine, Gaziantep, Turkey
[2]Department of Otorhinolaryngology, Private IMC Hospital, Mersin, Turkey
[3]Department of Otorhinolaryngology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey
[4]Department of Otorhinolaryngology, Adıyaman Training and Research Hospital, Adıyaman, Turkey

Corresponding Author: İsmail Aytaç

E-mail: dr.iaytac@gmail.com

Received Date: 18.04.2021

Accepted Date: 02.08.2021

How to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042.

ABSTRACT

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

Methods: This prospectively 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 fifty-five cases P16 (%76,4) expressions were detected to be positive, seventeen (23.6%) cases was negative. There was no statistically significant correlation between prognostic parameters and p16 expressions. However, a significant difference was detected between HPV positive and negative groups in regard to survival in oropharyngeal carcinoma.

Conclusion: Regarding disease management, it can consider of HPV positive oral cavity and oropharyngeal carcinomas as a separate group. HPV positive oral cavity and oropharyngeal carcinomas respond better to chemotherapy and radiotherapy than HPV negative cancers. Presence/absence of HPV 16 might be considered as a prognostic marker but its reliability has not yet been confirmed. In future clinical studies, cancer centers should classify head-neck patients in respect to HPV status. However, we must always emphasize that the best treatment for the cancer in which 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 at 5^{th} and 6^{th} decades (2). % 90 of the cancer appeared on Head-Neck region are squamous cell carcinomas. Oral cavity and oropharynx cancers are the most common cancers in all over the world and the second most common cancer in our country.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

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

HPV is a DNA group virus in the family of Papovaviridea in which two hundred different types have been identified. Molecular studies indicate that specific mechanisms play a role in HPV-induced carcinogenesis, and it has been thought of a relation 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 respiratory-digestive system (8). These patients' carcinoembryonic antigen levels increased, such a cellular immunosuppression may predispose to cancer. Methods used in virus detection are election microscope, immunohistochemically staining, hybridization techniques (Southern Blot, Dot Blot, and in situ hybridization) and "polymerase chain reaction" (PCR). This causal association between HPV and squamous cell carcinomas suggests that the presence of the virus may be a high-risk indicator. between HPV and squamous cell carcinomas. Brandwein et al. (9) reported that presence of HPV DNA in laryngeal tumors was associated with prognosis.

In the present study, we aimed to investigate HPV p16 presence in oral cavity and oropharynx carcinomas with histochemical methods. Expected benefits of this study is to demonstrate the relationship of HPV p16 with clinicopathologic parameters in oral cavity and oropharyngeal carcinomas, determining the behavior model of oral cavity and oropharynx cancers in advance and providing the most appropriate methods for treatment.

METHODS

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

The patients with oral cavity and oropharynx squamous cell carcinomas 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, a histopathology other than squamous cell carcinoma, previously treated and with additional malignancies were excluded from the study.

After receiving the detailed history of the patient who meet the above characteristics, head and neck examination was performed and 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;

- 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 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 microns 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. A staining of over 70% was considered positive. **Figure 1a, 1b** show the positive p16 light microscope image of squamous cell carcinoma (x100) and **Figure 2a, 2b** show the image of a p16 negative patient with the same disease.

Statistical analysis

SPSS 22.0 (IBM Corporation, Armonk, New York, United States) 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 in comparing two 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 from 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 $p < 0.05$ was considered significant.

RESULTS

This study included 72 patients diagnosed with non-lip oral cavity and oropharyngeal squamous cell carcinoma at Department of Otorhinolaryngology Gaziantep University Faculty of Medicine. Twenty-six 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 divided first into two 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%), in the oral cavity of 52 patients (72.3%). Distribution of the disease in cases according to localization shown in **Figure 3**.

Seventeen (85%) of the cases with oropharyngeal cancers were male and 3 (15%) were female. Twenty-nine (%55,8) of the patients with oral cavity cancer were male and twenty-three (%

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

44,2) were female. Oral cavity and oropharynx incidence were found statistically higher in male than female (p=0,028)

HPV p16 was found to be positive in 17 of 72 (23,6 %) patients included in the study. Nine of52 cases (17,3 %) with oral cavity cancer were found to have HPV p16 positivity, eight of 20 cases (40 %) with oropharynx cancer were found to have positivity. A statistical difference was not recorded between oral cavity and oropharynx cancer in terms of HPV positivity (P=0,063; **Figure 4**).

63 (87,5 %) patients were operated, 9 (12,5 %) patients underwent to chemotherapy and radiotherapy after biopsy and histopathological diagnosis of squamous cell carcinoma. 62 Surgical excision and neck dissection were simultaneously performed in 62 of the patients who operated, the only one 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=0,424), was a significant difference in terms of stage between HPV positive and negative groups in oral cavity cancers (p=0,017). HPV positive group in oral cavitary cancers was seen at an earlier stage (**Table1**).

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=0,240, Oropharynx p=1) (**Table2**).

However, 3-year survival rate of HPV positive group was statistically higher than HPV negative group (p=0,032).

When the difference between sex and 3-years survival rate is evaluated, although no significant difference found between male and female groups in terms of 3-year survival in oral cavity cancers (p=0,381), it was found between male and female groups in terms of 3-year survival in

oropharynx cancers (p=0,001). Three-year survival in oropharynx cancers was found to be significantly worse in women.

When three-year survival is evaluated between the patients operated and the patients underwent chemotherapy/radiotherapy in oral cavity and oropharynx, a significant difference recorded between operated patients and those who underwent chemotherapy/radiotherapy in both groups oral cavity and oropharynx cancers (Oral cavity cancers p=0,001, Oropharynx cancers p=0,016). Three-year survival rate of operated patients found statistically better in oral and oropharyngeal cancers.

When the relation 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=0,611), however, a significant difference found between patients with or without metastasis in oral cavity cancer (p=0,049), non-metastatic group's 3-year survival rate was found statistically higher than metastatic group.

When the relation between relapse and 3-years survival is evaluated, no significant difference found between the groups with or without relapse in oral cavity cancers (p=0,115), however, a significant difference between the groups with or without relapse found in oropharynx cancers (p=0,046), in which non-relapsing group was higher.

DISCUSSION

Incidence of head and neck region cancers less than 5 percent 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 50-60 (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-years survival rate T1-T2: 51%, T3-T4: 18%, (11) the importance of oral and oropharynx cancers is increasing even more. Although the 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 TNM classification. Lymph node metastasis is the most important parameter accepted.

However, even 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, recently, some researchers thought viral factors might be the reason of differences.

Many studies have shown that smoking and alcohol use are major, common risk factors for head and neck squamous cell carcinoma (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 squamous cell carcinoma (OPSCC). For the first time, Gillison et al. (14) have reported 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 play 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 of upper respiratory tract pathologies. However, the viral oncogenic effects have been better reported in the literature in the last three decades(18-20). HPV 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 result in a transformed phenotype. This data indicates that HPV play initiator role in the transformation of malignant. Immunohistochemical staining, hybridization techniques (Southern blot, dot blot and in situ hybridization) and polymerase chain reaction (PZR) 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 squamous cell carcinomas suggests that the presence of the virus may be a high risk for developing cancer. The high-risk subtypes of HPV are HPV 16, 18, 31, 33, 39, 45, 52, 58, and 69, play role in cervical and other anogenital cancers. HPV 6 and 11 are “low risk” types and rarely seen in malign lesions. They are mainly occurred in non-malign lesions.

In some studies, the reasons for HPV infections on 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 patient 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 a great many studies in literature that investigated HPV prevalence in head and neck cancers, which has been detected as 34,5 %. However, a wide range of 7 to 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 the method of immunohistochemically staining. 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 found high (40 %) in oropharynx cancers, which 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 % Verrucous carcinoma, and 46,5 % oral squamous cell carcinoma.

SahebJameeet al. (30) investigated the presence of HPV in the saliva of cases with oral squamous cell carcinoma (SCC) and control group with PCR method. HPV found to be positive in 40,9 %of SCC cases and 25% of control group. HPV 16 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 squamous cell carcinomas 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, 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 %) is in stage 1, 14 cases are in stage 2 (22,2 %), 13 cases is in stage 3 (20,6 %), 21 cases is 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 stage in oropharyngeal carcinomas has been recorded. However, there was a significant difference between HPV and stage 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 are 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.

HPV positive cancers' T stage is consistent with the literature but differs from the literature in 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.

HPV 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 was 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. HPV 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 word, our general purpose should be to provide high level of life quality and minimal treatment complication. In some studies, this type of treatment strategies seems to be possible for HPV induced cancers so that 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 in especially 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.

MAIN POINTS

-Oral cavity and oropharynx squamous cell cancers should be examined for HPV 16 positivity.

- Immunohistochemical examination is a suitable method in 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.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

REFERENCES

1. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127(12):2893-917.

2. Sturgis EM, Ang KK. The epidemic of HPV-associated oropharyngeal cancer is here: is it time to change our treatment paradigms? J Natl Compr Canc Netw 2011;9(6):665-73.

3. Gillison ML, D'Souza G, Westra W, Sugar E, Xiao W, Begum S, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. J Natl Cancer Inst 2008;100(6):407-20.

4. Marur S, D'Souza G, Westra WH, Forastiere AA. HPV-associated head and neck cancer: a virus-related cancer epidemic. Lancet Oncol 2010;11(8):781-9.

5. Kreimer AR, Clifford GM, Boyle P, Franceschi S. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. Cancer Epidemiol Biomarkers Prev 2005;14(2):467-75.

6. Shope RE, Hurst EW. Infectious Papillomatosis of Rabbits: With a Note on the Histopathology. J Exp Med 1933;58(5):607-24.

7. Carcopino X, Henry M, Olive D, Boubli L, Tamalet C. [Detection and quantification of human papillomavirus genital infections: virological, epidemiological, and clinical applications]. Med Mal Infect 2011;41(2):68-79.

8. Snijders PJF, Van den Brule AJC, Meijer CJLM, Walboomers JM. Papillomaviruses and cancer of the upper digestive and respiratory tracts. Curr Top Microbiol Immunol 1994;186:177-98.

9. Brandwein MS, Nuovo GJ, Biller H. Analysis of prevalence of human papillomavirus in laryngeal carcinomas. Study of 40 cases using polymerase chain reaction and consensus primers. Ann Otol Rhinol Laryngol 1993;102(4 Pt 1): 309-13.

10. Mendenhall WM, Tannehill SP, Hotz MA, Kásler M, Remenár E. Should chemotherapy alone be the initial treatment for glottic squamous cell carcinoma? Eur J Cancer 1999;35(9):1309-13.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

11. Taxy JB. Upper respiratory tract, in Anderson's Pathology. 10th ed. St. Louis: Mosby; 1996. p.1460-9.

12. Gnagy S, Ming EE, Devesa SS, Hartge P, Whittemore AS. Declining ovarian cancer rates in U.S. women in relation to parity and oral contraceptive use. Epidemiology 2000;11(2):102-5.

13. Shiboski CH, Schmidt BL, Jordan RC. Tongue and tonsil carcinoma: increasing trends in the U.S. population ages 20-44 years. Cancer 2005;103(9):1843-9.

14. Gillison ML, Koch WM, Capone RB, Spafford M, Westra WH, Wu L, et al. Evidence for a causal association between human papillomavirus and a subset of head and neck cancers.J Natl Cancer Inst 2000;92(9):709-20.

15. Gillison ML. Human papillomavirus-associated head and neck cancer is a distinct epidemiologic, clinical, and molecular entity. Semin Oncol 2004;31(6):744-54.

16. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. Cancer 2010;116(9):2166-73.

17. Frega A, Manzara F, Schimberni M, Guarino A, Catalano A, Bianchi P, et al. Human papilloma virus infection and cervical cytomorphological changing among intrauterine contraception users. Eur Rev Med Pharmacol Sci 2016;20(17):3528-34.

18. Adelstein DJ, Ridge JA, Gillison ML, Chaturvedi AK, D'Souza G, Gravitt PE, et al. Head and neck squamous cell cancer and the human papillomavirus: summary of a National Cancer Institute State of the Science Meeting, November 9-10, 2008, Washington, D.C. Head Neck 2009;31(11):1393-422.

19. Boshart M, Gissmann L, Ikenberg H, Kleinheinz A, Scheurlen W, zur Hausen H. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J 1984;3(5):1151-7.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

20. Löning T, Meichsner M, Milde-Langosch K, Hinze H, Orlt I, Hörmann K, et al. HPV DNA detection in tumours of the head and neck: a comparative light microscopy and DNA hybridization study. ORL J Otorhinolaryngol Relat Spec 1987;49(5):259-69.

21. Fakhry C, Westra WH, Li S, Cmelak A, Ridge JA, Pinto H, et al. Improved survival of patients with human papillomavirus-positive head and neck squamous cell carcinoma in a prospective clinical trial. J Natl Cancer Inst 2008;100(4):261-9.

22. Ragin CC, Taioli E. Survival of squamous cell carcinoma of the head and neck in relation to human papillomavirus infection: review and meta-analysis. Int J Cancer 2007;121(8):1813-20.

23. Bussu F, Sali M, Gallus R, Vellone VG, Zannoni GF, Autorino R, et al. HPV infection in squamous cell carcinomas arising from different mucosal sites of the head and neck region. Is p16 immunohistochemistry a reliable surrogate marker? Br J Cancer 2013;108(5):1157-62.

24. Rietbergen MM, Snijders PJ, Beekzada D, Braakhuis BJ, Brink A, Heideman DA, et al. Molecular characterization of p16-immunopositive but HPV DNA-negative oropharyngeal carcinomas. Int J Cancer 2014; 134(10):2366-72.

25. Morshed K, Polz-Dacewicz M, Rajtar B, Szymanski M, Ziaja-Soltys M, Golabek W. [The prevalence of E6/E7 HPV type 16 in laryngeal cancer and in normal mucosa]. Pol Merkur Lekarski 2005;19(111):291-3.

26. D'Souza G, Kreimer AR, Viscidi R, Pawlita M, Fakhry C, Koch WM, et al. Case-control study of human papillomavirus and oropharyngeal cancer. N Engl J Med. 2007;356(19):1944-56.

27. Frisch M, Biggar RJ. Aetiological parallel between tonsillar and anogenital squamous-cell carcinomas. Lancet 1999;354(9188):1442-3.

28. Hemminki K, Dong C, Frisch M. Tonsillar and other upper aerodigestive tract cancers among cervical cancer patients and their husbands. Eur J Cancer Prev 2000;9(6):433-7.

29. Miller CS, Johnstone BM. Human papillomavirus as a risk factor for oral squamous cell carcinoma: a meta-analysis, 1982-1997. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;91(6):622-35.

30. SahebJamee M, Boorghani M, Ghaffari SR, AtarbashiMoghadam F, Keyhani A. Human papillomavirus in saliva of patients with oral squamous cell carcinoma. Med Oral Patol Oral Cir Bucal 2009;14(10):e525-e8.

31. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363(1):24-35.

32. Huang SH, Perez-Ordonez B, Liu FF, Waldron J, Ringash J, Irish J, et al. Atypical clinical behavior of p16-confirmed HPV-related oropharyngeal squamous cell carcinoma treated with radical radiotherapy. Int J Radiat Oncol Biol Phys 2012;82(1):276-83.

33. Goldenberg D, Begum S, Westra WH, Khan Z, Sciubba J, Pai SI, et al. Cystic lymph node metastasis in patients with head and neck cancer: An HPV-associated phenomenon. Head Neck 2008;30(7):898-903.

34. Lim MY, Dahlstrom KR, Sturgis EM, Li G. Human papillomavirus integration pattern and demographic, clinical, and survival characteristics of patients with oropharyngeal squamous cell carcinoma. Head Neck 2016;38(8):1139-44.

35. Chaturvedi AK. Epidemiology and clinical aspects of HPV in head and neck cancers. Head Neck Pathol 2012;6 Suppl 1:16-24.

TABLES

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

Table 1. Comparison the relationship between HPV and stages.

		STAGES					
		I	II	III	IV	Total	P Value
	HPV	n (%)	n (%)	n (%)	n (%)		
Oropharynx	-	1 (14,3)	2 (28,6)	2 (28,6)	2 (28,6)	7 (100)	0,424
	+	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)	0,017
	+	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)	0,330
	+	2 (12,5)	6 (37,5)	3 (18,8)	5 (31,3)	16 (100)	

Pearson Chi-Square Test (Monte Carlo)

Table 2. Comparison 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 Value		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 Value		1		1		1	

Fisher Exact Test (Exact)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

Figure Legends

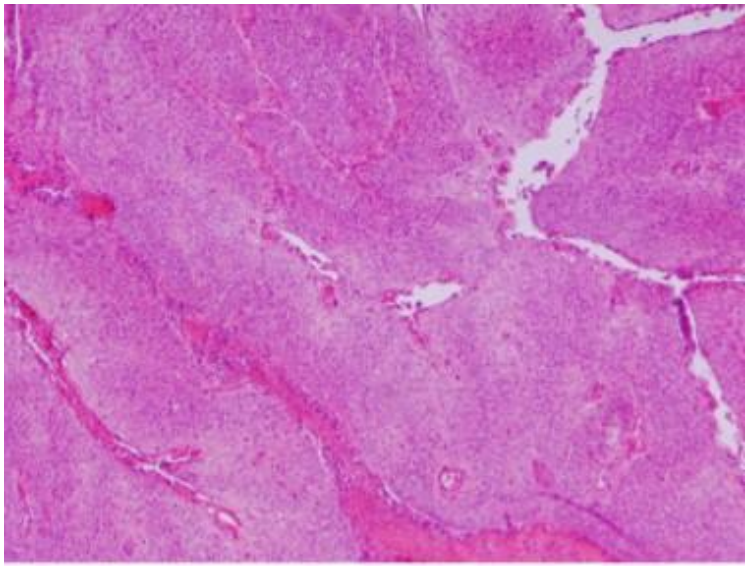

Figure 1a. Histopathological view of squamous cell carcinoma (H-Ex100)

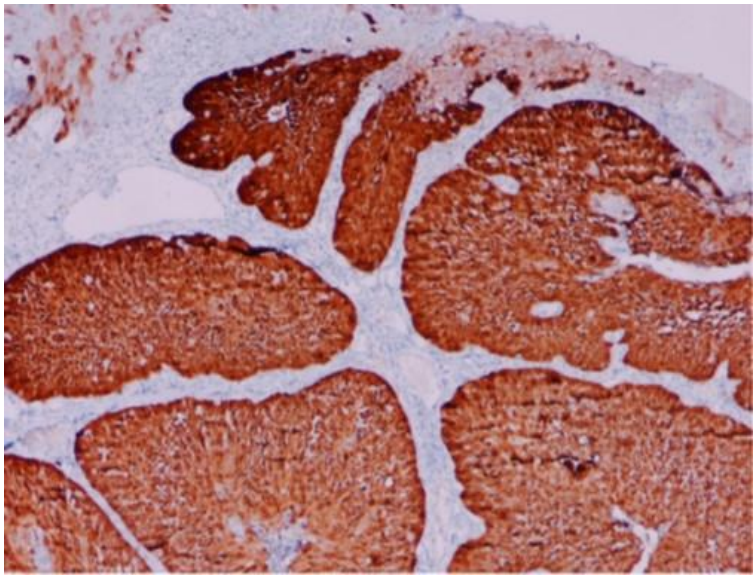

Figure 1b. Positive p 16 image of same patient (x100)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

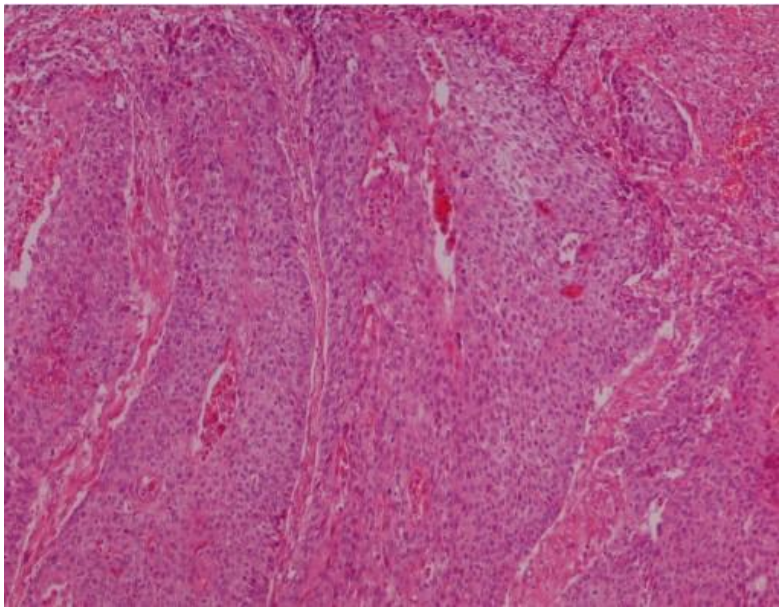

Figure 2a. Histopathological view of squamous cell carcinoma (H-Ex100)

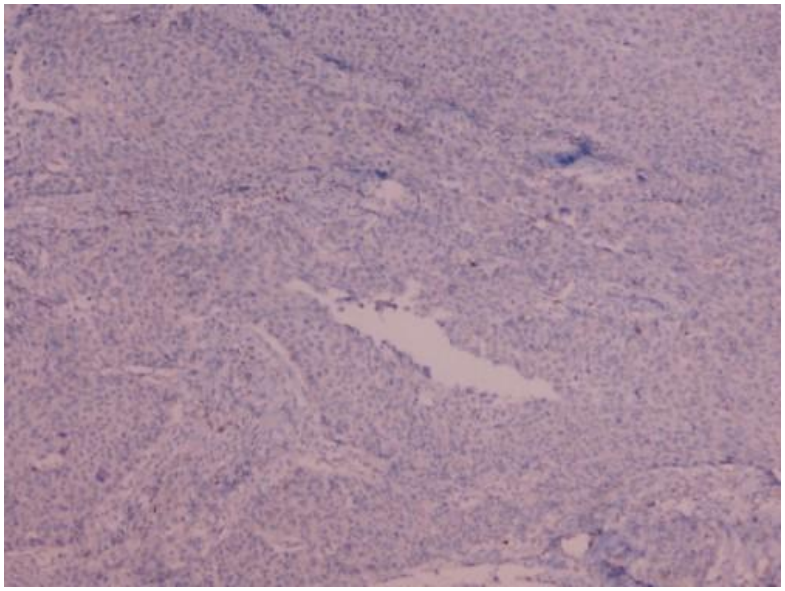

Figure 2b. Negative p 16 image of same patient (x100)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

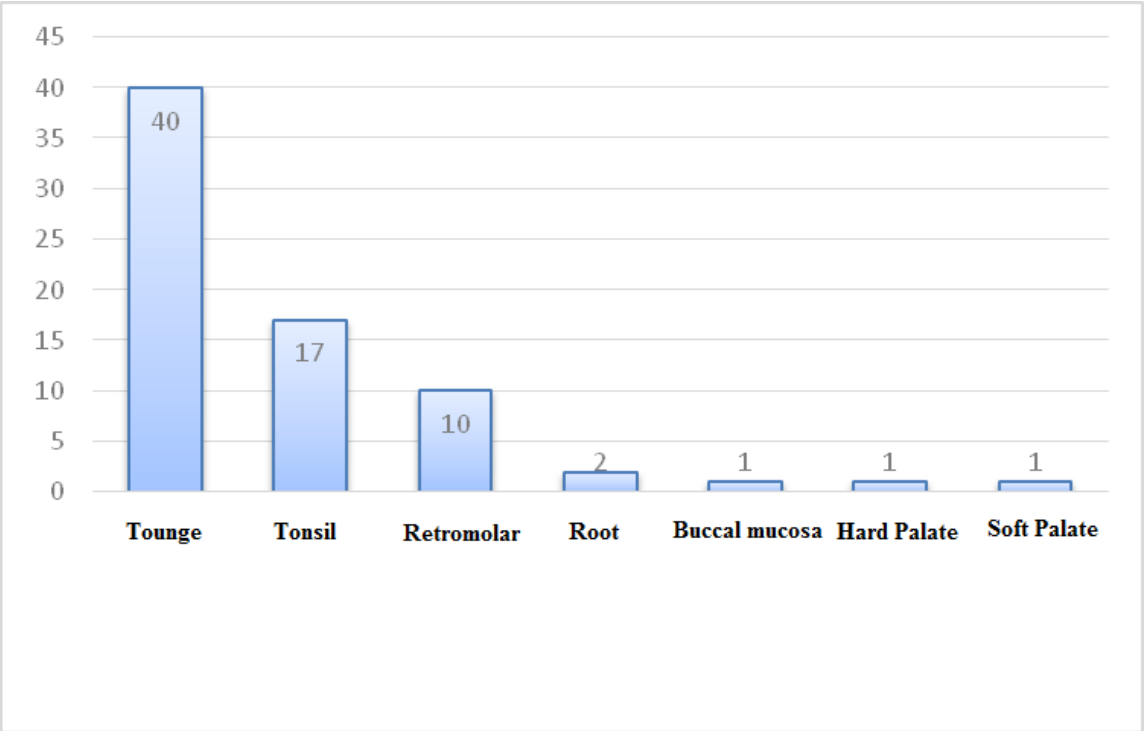

Figure 3. Distribution of cases based on localization.

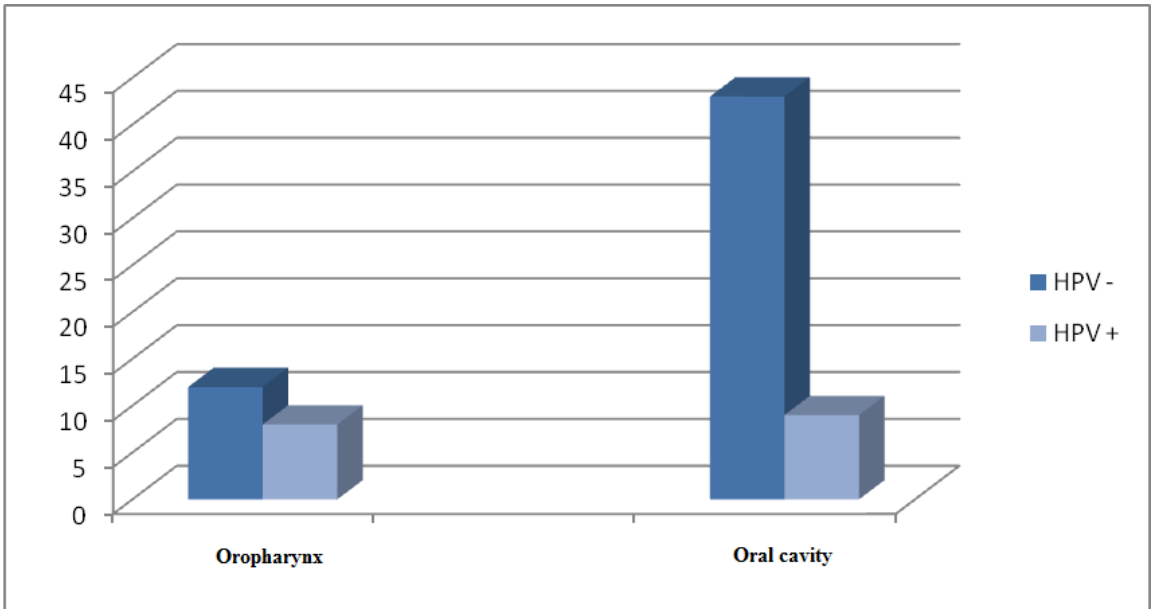

Figure 4. Distribution of Oral Cavity and Oropharynx Cancers based on HPV p16 Staining.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please how to cite: Tümüklü K, Çelenk F, Aytaç İ, Kurt E, Kanlikama M. Exploring The Role of Hpv 16 in Squamous Cell Cancers of Oral Cavity and Oropharynx. Eur J Ther 2021; DOI: 10.5152/EurJTher.2021.21042. - Available online at https://eurjther.com/EN

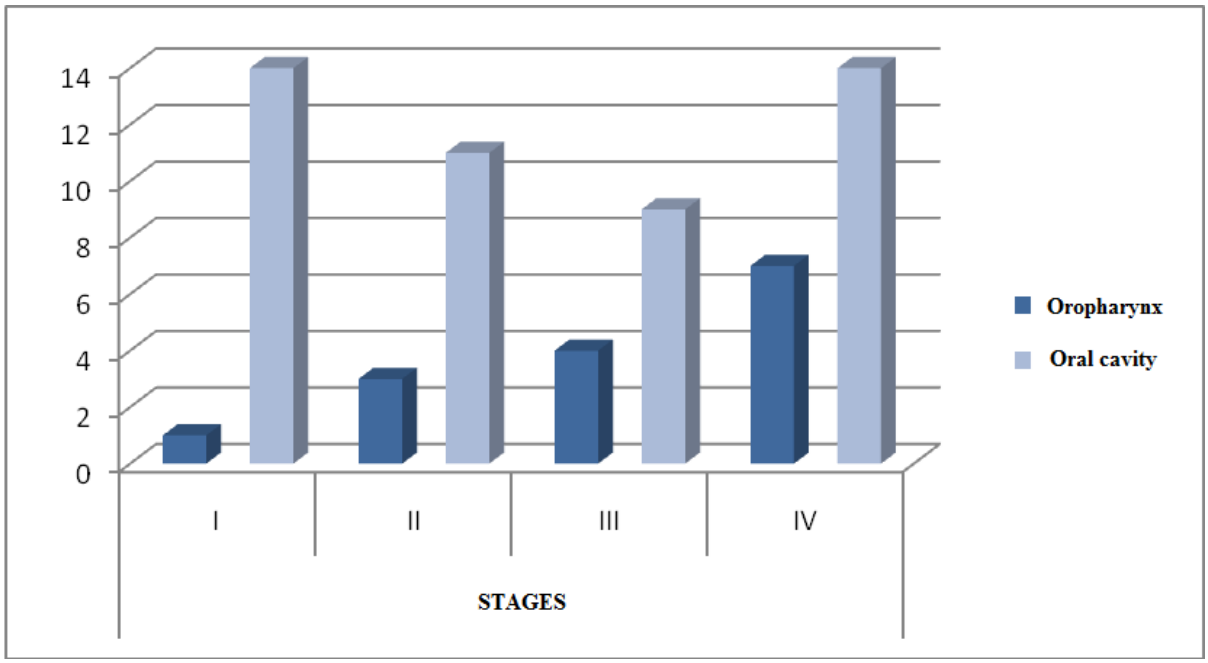

Figure 5. Distribution of Oral Cavity and Oropharynx Cancer in Terms of Stage.