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ORIGINAL ARTICLE

Detection of HPV DNA in Esophageal Lesions: a Cross-Sectional Study

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SUMMARY

Background: **Several studies have documented human papillomavirus (HPV) in extra-cervical tumors. We aimed to detect HPV type 16 and HPV other than type 16 (OT-16) DNA in esophageal papilloma and esophagus squamous cell carcinoma (ESCC) samples and to compare clinicopathological features of HPV positive and negative patients.**
Methods: **Materials were obtained from a tertiary care public hospital and studied in an university hospital for this cross-sectional study. Seventy-six tissue samples (50 papilloma and 26 ESCC) were included. After deparaffinization by xylene and DNA extraction by phenol chloroform-isoamyl-alcohol, 76 samples were studied with a G6PDH control kit. Forty-four papilloma and 21 ESCC samples with enough tissues were studied for HPV DNA. HPV OT-16 DNA and HPV type 16 were detected by real time-polymerase chain reaction.**
Results: **Twelve (27.3%) and one (2.3%) of the papilloma samples were HPV type 16 and other than type 16 positive, respectively. Eleven (52.4%) and one (4.8%) of ESCC samples were HPV type 16 and mixed type positive, respectively.**
Conclusions: **We suggest that HPV infection is common in esophageal papilloma and ESCC. Due to the well-known association of HPV with premalignant and malignant conditions, follow-up of these patients accompanied by HPV should be implemented.**
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KEY WORDS

human papillomavirus, papilloma, esophageal neoplasms, real-time polymerase chain reaction

INTRODUCTION

Exposure to infectious agents - such as bacterial or viral infections - sometimes initiates the development of many neoplasms. Some viruses are seen as a cause of development of carcinogenesis. Nearly one-fifth of the cancers occurring in the human population are connected with infection as a causative factor. The causative

role of human papillomavirus (HPV) in human cancers is evident [1]. HPVs cause benign, precancer or malignant lesions of the genital and anal areas, as well as head and neck cancers [2]. Overall, 630,000 cancer cases (4.5% of all cancer cases) are attributable to HPV every year, representing a major threat to public health worldwide [3]. More than 100 types of HPV have been identified and type 16 and 18 are the most prevalent high-risk types related with cancers [4].

After the evidence of the causative role of HPV in cervical cancer in the early 1980s, HPV was found in other regions of the human body. Scientists were directed to search for HPV in other parts of the body. Although HPV is a well-established risk factor for cervical, urogenital, and oropharyngeal cancers, there is not a clear relationship with all types of the cancers including the gastrointestinal tract [5].

Esophageal squamous papilloma is a relatively rare, benign, squamous epithelial tumor related with HPV infection and hyper-regenerative response of the mucosa to chemical and mechanical irritations [6]. Syrjanen was the first who demonstrated the presence of HPV antigens in squamous papillomas, and HPV infection has been considered as one of the etiological factors of squamous papillomas [7].

Esophageal cancer is the eighth most common cancer and the sixth leading cause of cancer-related death worldwide [8]. Overall survival rates range between 5% and 16%. In the highest risk area, stretching from northern Iran through the central Asian republics to North-Central China, often called the "esophageal cancer belt", 90% of esophageal cancers are squamous cell carcinomas (ESCC). The etiology of esophageal cancer remains unclear. Risk factors such as use of tobacco and alcohol, lack of nutrition, and some chemical factors were found. Infectious agents such as HPV have also been suggested as direct carcinogens or promoters in esophageal carcinogenesis [8].

The aim of the present study was to determine whether HPV, especially type 16, is involved in Turkish patients with esophageal lesions including esophageal papillomas and ESCC and to compare the clinicopathological differences between HPV-positive and negative cases.

MATERIALS AND METHODS

Ethical considerations

The Ethical Review Board of Yuksek Ihtisas Hospital approved this study (11.06.2014/0012).

Study group

This was a retrospective cross-sectional study that used Formalin Fixed Paraffin Embedded tissue material from patients diagnosed with esophageal lesions who attended to Yuksek Ihtisas Hospital in Ankara, Turkey. The study was performed on 76 patient samples with esophageal papilloma and ESCC submitted to the Department of Pathology of Yuksek Ihtisas Hospital.

Histopathologic examination

The histopathological type of the papillomas and cancer tissues were rereviewed and recorded as papilloma and squamous cell carcinoma in esophageal samples. Clinical stages (T stages were defined as T1, T2, T3, and T4 and N stages as N0, N1, and N2) were defined for adenocarcinomas and squamous cell carcinomas. In addition, cytology differentiation was classified as poor, moderate, and well in ESCC samples.

Real-time-polymerase chain reaction (PCR) analysis

DNA extraction

After deparaffinization by xylene, the samples were extracted by phenol chloroform isoamyl alcohol method.

Control tests

Paraffin-embedded cell lines of Caski were used as a positive control for extraction as well as for PCR of HPV. Paraffin-embedded smooth muscle specimens were used as negative controls. A positive internal control for glucose-6-phosphatase dehydrogenase (G6PDH control kits, Eurogentec, Belgium) was used to determine the adequacy of DNA retrieval. Specimens with undetectable G6PDH were considered as not having enough DNA for HPV detection and excluded.

DNA amplification

The nested real-time PCR (rt-PCR) method was used for the analysis of HPV DNA (any type) and HPV-16 positivity. The MY09/11 primer set was used for PCR amplification following the deparaffinization and extraction of the DNA. Real-time nested amplifications of MY09/11 products were done by GP5+/GP6+ primers and a cyanine-5-labeled HPV-16 DNA-specific probe. Real-time PCR product analysis was done by melting curve analysis on LightCycler software version 3.5.3 (LC 2.0, Roche Diagnostics, Germany). Melting peaks of 78°C to 82°C detected the HPV DNA in the sample. Probe melting peaks of positive samples were analyzed in the same run, and HPV-16-positive samples yielded peaks around 68°C ± 2°C.

Statistical analysis

HPV positive and negative cases were compared regarding gender, age, site of papillomas and stages of ESCC by chi-square and Fisher's exact tests of SPSS version 14. Statistical significance was defined as $p < 0.05$.

RESULTS

Of the 65 G6PDH positive samples, 44 (68%) and 21 (32%) were esophageal papillomas and ESCC, respectively. A total of 32 (50.7%) and 33 (49.3%) of the patients were male and female, respectively, and the mean age of patients was 52.7 years (range 23 - 78 years). HPV type 16 and other than type 16 were detected in 12 (27.3%) and one (2.3%) of squamous papilloma sam-

Table 1. HPV DNA results of papilloma and ESCC patients.

	HPV type 16 (+)		HPV other than type 16 (+)		Mixed type (+)		HPV (-)	
	n	%	n	%	n	%	n	%
Papilloma	12	27.3	1	2.3	-	-	21	70.4
ESCC	11	52.3	-	-	1	4.8	9	42.9

HPV - human papillomavirus, DNA - deoxyribonucleic acid, ESCC - esophageal cancers are squamous cell carcinomas.

Table 2. Clinicopathological features of esophageal papilloma patients.

	HPV type 16 (+)		HPV other than type 16		HPV (-)		Total		p-value
	n	%	n	%	n	%	n	%	
Gender									
Female	8	34.8	-	-	15	65.2	23	52.3	0.320
Male	4	19	1	4.8	16	76.2	21	47.7	
Age									
≤ 60	8	25	1	3.1	23	71.9	32	72.7	0.729
≥ 61	4	33.3	-	-	8	66.7	12	27.3	
Anatomic localization									
Upper third	1	10	-	-	9	90	10	22.7	0.173
Middle third	9	37.5	-	-	15	62.5	24	54.6	
Lower third	2	20	1	10	7	70	10	22.7	

HPV - human papillomavirus.

Table 3. Clinicopathological features of esophageal squamous cell carcinoma patients.

	HPV type 16 (+)	Mixed type (+)		HPV (-)		Total		p-value
	%	n	%	n	%	n	%	
Gender								
Female	60	-	-	4	40	10	47.6	0.561
Male	45.5	1	9	5	45.5	11	52.4	
Age								
≤ 60	63.6	-	-	4	36.4	11	52.4	0.389
≥ 61	40	1	10	5	50	10	47.6	
Stage								
Stage I	25	1	25	2	50	4	19	0.391
Stage IIA	60	-	-	4	40	10	47.6	
Stage IIB	75	-	-	1	25	4	19	
Stage III	33.7	-	-	2	66.3	3	14.4	
Differentiation								
Poorly differentiated	50	-	-	4	50	8	38	0.791
Moderately differentiated	55.6	1	11.1	3	33.3	9	43	
Well differentiated	50	-	-	2	50	4	19	

ples, respectively. HPV type 16 and mixed type were detected in 11 (52.4%) and one (4.8%) of ESCC samples, respectively (Table 1).

The comparison of clinicopathological features (gender, age, and anatomic localization) among esophageal papilloma patients are summarized in Table 2 and there was not a statistically significant difference between the HPV positive and negative group (Table 2).

The comparison of clinicopathological features (gender, age, stage, and differentiation) among ESCC patients are summarized in Table 3. Regarding gender, age, stage, and differentiation there was no statistically significant difference between the HPV positive and negative groups (Table 3).

DISCUSSION

Although the role of HPV in esophageal lesions is not as clearly understood as in cervical cancer, a number of studies have provided evidence on the presence of HPV in esophageal papillomas and ESCC [9]. Esophageal papilloma is a considerable risk factor of esophageal carcinoma which is the sixth most common cause of cancer-related mortality in the world and has poor prognosis when detected at advanced states. Therefore, it is important to diagnose these lesions in the esophagus before their neoplastic transformation [10]. The differences in the results of the studies may be due to differences in race, living habits, environmental factors that lead to HPV infection or differences in research design, detection means, methods, and statistical analysis [11]. In this study more than half of the patients with ESCC and one third of the patients with squamous papilloma were positive for HPV. Because of the rarity, the number of cases was not adequate to draw a definitive conclusion regarding the role of HPV in the etiology of esophageal papillomas. However, it can be seen that patients with ESCC have higher HPV positivity rate than patients with squamous papilloma.

Depending upon the methods, the detection rates of HPV in esophageal papillomas varies from 0% to 87.5% [12]. Similarly, prevalent types of HPV in esophageal papillomas also differ among studies. Some studies reported 6 and 11 as major types of HPV, while other studies reporting the detection of cancer associated HPV types especially type 16 [12,13]. Our study, also detected mainly type 16. Therefore, we suggest high risk HPV types can also be a possible etiological factor of papillomas.

Although a female predominance was observed in our study in HPV positive samples, it was not statistically significant. Tiftikçi et al. [6] evaluated 38 papilloma cases, 7 (19%) of which were positive for HPV DNA; 2/7 of these positive cases were from female patients. (5 males and 2 females) and 4/7 were the highly oncogenic genotypes.

Bohn et al. [12] studied HPV DNA by PCR in papilloma samples and HPV DNA was positive in 12 (85.7%) of 14 cases and 9/12 (75%) of the positive cases were younger than 60 years. Like the study of Bohn et al. [12], we found that 8/12 (66.6%) of the positive samples were from patients younger than 60 years.

In our study, more than half of the papillomas and 70% of the HPV-positive papillomas were located in the middle esophagus. Tiftikçi et al. [6] also found that 5/7 of HPV positive papillomas were located in the middle esophagus. This may be related to the high presence of papillomas in the middle esophagus. Although statistically not significant, these results indicate that the middle esophagus might represent a favorable environment for HPV infection.

Recent findings [14,15] raise the possibility that HPV is involved in esophageal carcinogenesis. Studies about esophageal cancer and HPV relationship were usually performed in areas where esophageal cancer is endemic. Turkey is not an endemic area for esophageal cancer, and it is not among the most prevalent 10 cancers according to the Turkish Ministry of Health (http://kanser.gov.tr/Dosya/ca_istatistik/2014-RAPOR._uzun.pdf).

A study from Iran reported that HPV DNA was detected in esophageal specimens of 16 out of the 51 ESCC cases (31.4%) [16]. Being next to Iran, the Esophageal Cancer belt has the world's highest areas of incidence, leading us think about the association of HPV and ESCC. Furthermore, in endemic areas for esophageal cancer such as Iran and China, studies could not find any association between HPV infection and ESCC [17-19]. To our knowledge, two studies were performed on the relationship between esophageal cancer and HPV in Turkey; Erol et al. [20] found that 1/4 (25%) of the esophagus cancer samples were HPV-DNA positive and Turkay et al. [21] found that five (9.6%) of 52 tumor samples, 3 squamous cell carcinomas (3/33 cases) and 2 adenocarcinomas (2/19 cases), were HPV-DNA-positive and subtype analysis could be performed in four HPV-DNA-positive cases, of which three were HPV type-39 and one was type-16. Twenty-one ESCC samples were included in our study and more than half were HPV DNA positive (57.1%). Globally, there are controversial studies about the HPV-esophageal cancer relationship. For example; Schäfer et al. [22] concluded that HPV infectivity seems to play only a minor role in esophageal cancer since HPV DNA was found in about 9% of ESCC patients. In a meta-analysis by Wang et al. [23], HPV infection rate in the ESCC group was 46.5%, while HPV infection rate in the control group was 26.2% indicating that HPV infection and the incidence of ESCC are closely associated. Dabrowski et al. [24] identified HPV DNA in 28 of 56 patients (50%) and found that the occurrence of HPV in ESCC patients was significantly higher than in the controls (28 of 56 (50%) versus 4 of 35 (11.4%), $p < 0.001$).

There are different studies evaluating different types of HPV in esophageal carcinoma. A study from China found that the prevalence of high-risk HPV types increased significantly during the progression of esophageal carcinogenesis [25]. High risk HPV types are more

frequently observed in esophageal carcinoma samples. In a multi-local study from China and the United States, more than half of the tested samples were HPV DNA positive [26]. In a systematic review, 23% and 10% of the patients with esophageal cancer and control group, respectively, were positive for HPV. The most common type was HPV 16 (40.9%) followed by other high-risk types such as HPV 18, 31, 33, 45, and 52 [27]. A study from China [28] found supportive results where about half of the cases of ESCC were of HPV type 16. Twenty-one ESCC samples were included in our study and more than half were HPV DNA positive and, except one, all were of type 16.

Esophageal cancer rates are typically 2 - 4 times higher among males than females. In Latin America, although statistically not significant, the male:female ratio in HPV positive ESCC cases increased two-fold in comparison with HPV negative cases [29]. In contrast, male and female ratio was equal in Iran but the gender-ratio between HPV positive and negative cases was not statistically significant [30]. In contrast, Dabrowski et al. [24] found a higher prevalence of HPV in female patients. In our study half of the positive cases were from male patients, and regarding gender, there was no statistically significant difference between HPV positive and negative groups.

The association between HPV infection and age was also studied. In Sweden, Löfdahl et al. [31] found that 75% (15/20) of HPV positive ESCC cases were older than 60 years. In contrast, we found that 7/12 (58.3%) HPV positive ESCC patients were younger than 60 years.

In Kashmir, a higher occurrence of HPV infection was detected in advanced stage of cancer by Hussain et al. [32]. We detected the highest positive rate among Stage IIA patients, it may be due to the large number of patients in this group. Similar to our study, Ding et al. [29] found that HPV16 was 47% (8/17) positive in ESCC, and there were no correlations between HPV16 infection and TNM stage.

Comparing the differentiation of the tumor and HPV relationship, we could not demonstrate a statistically significant difference between HPV and the degree of cell differentiation in our study but Tornesello et al. [33] detected a statistically significant high frequency of HPV infection in well and moderately differentiated grades compared to the poorly differentiated group.

There are some limitations of this study; one of the most important limitations is the lack of a control group due to ethical problems in obtaining normal esophageal tissues. We also do not have any data about the patients' nutrition habits or use of alcohol/tobacco which are one of the main risk factors of gastrointestinal malignancies. However, these limitations do not invalidate the results.

CONCLUSION

The present study is the first one reporting the presence of HPV and high-risk HPV type 16 both in esophageal papillomas and ESCC by real time PCR in Turkey. The relationship of HPV infection with risk factors of these lesions such as gender, age, site of esophageal papillomas, TNM stage and differentiation for malignancies were also evaluated. We believe that our results may provide important contributions to controversies about the relationship of HPV-esophageal lesions. We concluded that follow-up of patients with esophageal lesions accompanied by HPV should be implemented.

Declaration of Interest:
There is no conflict of interest.

References:

1. Zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. Virology 2009;384(2):260-5 (PMID: 19135222).

2. Mirghani H, Sturgis EM, Auperin A, Monsonego J, Blanchard P. Is there an increased risk of cancer among spouses of patients with an HPV-related cancer: A systematic review. Oral Oncol 2017;67:138-45 (PMID: 28351568).

3. Serrano B, Brotons M, Bosch FX, Bruni L. Epidemiology and burden of HPV-related disease. Best Pract Res Clin Obstet Gynaecol 2018;47:14-26 (PMID: 29037457).

4. Malekpour Afshar R, Deldar Z, Mollaei HR, Arabzadeh SA, Iranpour M. Evaluation of HPV DNA positivity in colorectal cancer patients in Kerman, Southeast Iran. Asian Pac J Cancer Prev 2018;19(1):193-8 (PMID: 29373913).

5. Omrani-Navai V, Alizadeh-Navaei R, Yahyapour Y, et al. Human papillomavirus and gastrointestinal cancer in Iranian population: A systematic review and meta-analysis. Caspian J Intern Med 2017;8(2):67-85 (PMID: 28740634).

6. Tiftikçi A, Kutsal E, Altıok E, et al. Analyzing esophageal squamous cell papillomas for the presence of human papilloma virüs. Turk J Gastroenterol 2017;28(3):176-8 (PMID: 28316321).

7. Syrjänen KJ. Two Landmark Studies Published in 1976/1977 Paved the Way for the Recognition of Human Papillomavirus as the Major Cause of the Global Cancer Burden. Acta Cytol 2017; 61(4-5):316-37 (PMID: 28693008).

8. Guo L, Liu S, Zhang S, et al. Human papillomavirus-related esophageal cancer survival: A systematic review and meta-analysis. Medicine (Baltimore) 2016;95(46):e5318 (PMID: 27861358).

9. Bognár L, Hegedűs I, Bellyei S, et al. Prognostic role of HPV infection in esophageal squamous cell carcinoma. Infect Agent Cancer 2018;13:38 (PMID: 30519280).

10. Sieroń-Stołtny K, Kwiatek S, Latos W, et al. Autofluorescence endoscopy with ''real-time'' digital image processing in differential diagnostics of selected benign and malignant lesions in the oesophagus. Photodiagnosis Photodyn Ther 2012;9:5-10 (PMID: 22369723).

11. Syrjänen K. Geographic origin is a significant determinant of human papillomavirus prevalence in oesophageal squamous cell carcinoma: systematic review and meta-analysis. Scand J Infect Dis. 2013;45(1):1-18 (PMID: 22830571).

12. Bohn OL, Navarro L, Saldivar J, Sanchez-Sosa S. Identification of human papillomavirus in esophageal squamous papillomas. World J Gastroenterol 2008;14(46):7107-11 (PMID: 19084918).

13. Pantham G, Ganesan S, Einstadter D, Jin G, Weinberg A, Fass R. Assessment of the incidence of squamous cell papilloma of the esophagus and the presence of high-risk human papilloma virus. Dis Esophagus 2017;30(1):1-5 (PMID: 27001250).

14. Guo F, Liu Y, Wang X, et al. Human papillomavirus infection and esophageal squamous cell carcinoma: a case-control study. Cancer Epidemiol Biomarkers Prev 2012;21(5):780-5 (PMID: 22 337534).

15. Gupta N, Barwad A, Rajwanshi A, Kochhar R. Prevalence of human papilloma virus in esophageal carcinomas: a polymerase chain reaction-based study. Acta Cytol 2012;56(1):80-4 (PMID: 22236750).

16. Yahyapour Y, Sadeghi F, Alizadeh A, Rajabnia R, Siadati S. Detection of Merkel Cell Polyomavirus and Human Papillomavirus in Esophageal Squamous Cell Carcinomas and Non-Cancerous Esophageal Samples in Northern Iran. Pathol Oncol Res 2016; 22(4):667-72 (PMID: 26879397).

17. Noori S, Monabati A, Ghaderi A. The prevalence of human papilloma virus in esophageal squamous cell carcinoma. Iran J Med Sci 2012;37(2):126-33 (PMID: 23115442).

18. Haeri H, Mardany O, Asadi-Amoli F, Shahsiah R. Human papilloma virus and esophageal squamous cell carcinoma. Acta Med Iran 2013;51(4):242-5 (PMID: 23690103).

19. Koshiol J, Wei WQ, Kreimer AR, et al. No role for human papillomavirus in esophageal squamous cell carcinoma in China. Int J Cancer 2010;127(1):93-100 (PMID: 19918949).

20. Erol D, Bulut Y, Yüce H, Ozercan IH. [Investigation of the presence of human papillomavirus DNA in various gastrointestinal carcinoma samples]. Mikrobiyol Bul 2009;43(2):259-68 (PMID: 19621611).

21. Türkay DÖ, Vural Ç, Sayan M, Gürbüz Y. Detection of human papillomavirus in esophageal and gastroesophageal junction tumors: A retrospective study by real-time polymerase chain reaction in an instutional experience from Turkey and review of literature. Pathol Res Pract 2016;212(2):77-82 (PMID: 26608416).

22. Schäfer G, Kabanda S, van Rooyen B, et al. The role of inflammation in HPV infection of the Oesophagus. BMC Cancer 2013; 13:185 (PMID: 23570247).

23. Wang J, Zhao L, Yan H, et al. A Meta-Analysis and Systematic Review on the Association between Human Papillomavirus (Types 16 and 18) Infection and Esophageal Cancer Worldwide. PLoS One 2016;11(7):e0159140 (PMID: 27409078).

24. Dabrowski A, Kwaśniewski W, Skoczylas T, et al. Incidence of human papilloma virus in esophageal squamous cell carcinoma in patients from the Lublin region. World J Gastroenterol 2012; 18(40):5739-44 (PMID: 23155315).

25. Liu HY, Zhou SL, Ku JW, et al. Prevalence of human papillomavirus infection in esophageal and cervical cancers in the high incidence area for the two diseases from 2007 to 2009 in Linzhou of Henan Province, Northern China. Arch Virol 2014;159(6): 1393-401 (PMID: 24385156).

26. Wang X, Tian X, Liu F, et al. Detection of HPV DNA in esophageal cancer specimens from different regions and ethnic groups: a descriptive study. BMC Cancer 2010;10:19 (PMID: 20078887).

27. Jalilvand S, Shoja Z, Hamkar R. Human papillomavirus burden in different cancers in iran: a systematic assessment. Asian Pac J Cancer Prev 2014;15(17):7029-35 (PMID: 25227786).

28. Ding GC, Ren JL, Chang FB, et al. Human papillomavirus DNA and P16(INK4A) expression in concurrent esophageal and gastric cardia cancers. World J Gastroenterol 2010;16(46):5901-6 (PMID: 21155014).

29. Herrera-Goepfert R, Lizano M, Akba S, Carrillo-García A, Becker-D'Acosta M. Human papilloma virus and esophageal carcinoma in a Latin-American region. World J Gastroenterol 2009; 15(25):3142-7 (PMID: 19575494).

30. Yahyapour Y, Shamsi-Shahrabadi M, Mahmoudi M, et al. Evaluation of human papilloma virus infection in patients with esophageal squamous cell carcinoma from the Caspian Sea area, north of Iran. Asian Pac J Cancer Prev 2012;13(4):1261-6 (PMID: 227993 15).

31. Löfdahl HE, Du J, Näsman A, et al. Prevalence of human papillomavirus (HPV) in oesophageal squamous cell carcinoma in relation to anatomical site of the tumour. PLoS One 2012;7(10): e46538 (PMID: 23077513).

32. Hussain S, Bharti AC, Salam I, et al. Transcription factor AP-1 in esophageal squamous cell carcinoma: alterations in activity and expression during human Papillomavirus infection. BMC Cancer 2009;9:329 (PMID: 19758438).

33. Tornesello ML, Monaco R, Nappi O, Buonaguro L, Buonaguro FM. Detection of mucosal and cutaneous human papillomaviruses in oesophagitis, squamous cell carcinoma and adenocarcinoma of the oesophagus. J Clin Virol 2009;45(1):28-33 (PMID: 19307150).