Int J Cancer Manag. 2020 July; 13(7):e105916. doi: 10.5812/ijcm.105916.

Published online 2020 July 20. **Research Article**

The Molecular Epidemiology of Herpes Simplex Virus Type 1 and 2 (HSV-1 and HSV-2) in Head and Neck Cancer (HNC)

Atiyeh Dabouian [1], Alireza Tabibzadeh [2], Ali Salimi-Jeda [2], Safoora Torkashvand [3], Mahshid Panahi [4], Fahimeh Safarnezhad Tameshkel [4], Farhad Zamani [4], Hossein Keyvani [4] and Mohammad Hadi Karbalaie Niya [2, 4, *]

[1] Minimally Invasive Surgery Research Center, Iran University of Medical Sciences, Tehran, Iran
[2] Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
[3] Emergency Department, Kasra Hospital, Tehran, Iran
[4] Gastrointestinal and Liver Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran

[*] *Corresponding author*: Assistant Professor in Medical Virology, Gastrointestinal and Liver Diseases Research Center, Iran University of Medical Sciences, Tehran, Iran; Department of Virology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran. Email: karbalai.mh@iums.ac.ir

Received 2020 June 01; **Revised** 2020 June 22; **Accepted** 2020 June 22.

Abstract

Background: Head and neck cancers (HNC) constitute the sixth common type of malignancies worldwide and can affect a wide range of anatomical regions. The role of the herpes simplex virus (HSV) in oral squamous cell carcinoma (OSCC) has been previously investigated.
Objectives: The objective of the current study was to evaluate the molecular epidemiology of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in patients with HNC.
Methods: A total of 156 patients with HNC were chosen including 90 biopsies and 66 formalin-fixed paraffin-embedded (FFPE) samples. HNC was confirmed and affected different anatomical regions. HSV detection was performed by a polymerase chain reaction (PCR) and HSV typing was assessed by a multiplex PCR.
Results: The 156 HNC specimens included 35 (22.4%) larynx, 29 (18.6%) tongue, 6 (3.8%) glands (parotid and tonsil), 12 (7.6%) nasopharynx, 9 (5.7%) pharynx, 33 (21%) vocal cord, 3 (1.9%) palatine, glottis 17 (10.8%), nasal 2 (1.3%), mandibular 4 (2.5%), lip 4 (2.5%), neck 1 (0.6%), and face 1 (0.6%). The mean ± SD of the patients' age was 60.3 ± 12.65, where 121 (77.1%) were male. The HSV was detected in 4 (2.6%) samples and 75% of HSV positive samples were HSV-1.
Conclusions: The rate of HSV infection in Iranian patients with HNC was 2.6% with the majority being HSV-1 (75%). As a preliminary study in Iranian patients with HNC, cancer location was not statistically significant. Further investigations are needed to assess the role of HSV in HNC.

Keywords: Head and Neck Cancers (HNC), Squamous Cell Carcinoma, Herpes Simplex Virus (HSV)

1. Background

Malignant diseases are a major health issue worldwide and the third cause of mortality in Iran (1, 2). Head and neck cancers (HNC) are the sixth common type of malignancies worldwide (1, 3). Head and neck squamous cell carcinoma (HNSCC) can affect a wide range of anatomical regions including oral cavity, nasal, nasopharynx, oropharynx, paranasal sinuses, larynx, thyroid, and parotid glands (2, 4, 5). The annual incidence rate of HNSCCs is 690000 new cases and the death rate is 380000 worldwide (2, 6). Further, there are 40000 new cases and 7890 death in the USA (7). HNSCC claims 21% of male cancers and 11% of female cancers in western Asia, India, and Pakistan (8). Oral cancer is one of the crucial HNSCCs due to its poor prognosis (9). There are 274000 new cases of oral squamous cell carcinoma (OSCC) worldwide annually. HNCs incidence in Iran is estimated 46.3 cases per 100,000 people (49.5 in men and 43 in women) (2). The majority of OSCC patients are older than 50 years old (10). Tobacco and alcohol consumption are known as major risk factors in OSCC tumorigenesis (11).

Different genetic and environmental factors such as tobacco, alcohol consumption, viral infections, and poor oral hygiene are associated with HNSCC (12-15). Different studies have shown the presence of DNA viruses such as herpes simplex virus (HSV), Epstein-Barr virus (EBV), and human papillomavirus (HPV) in oral dysplasia and OSCC specimens (16-19). Also, the role of the EBV and HPV in OSCC, na-

sopharyngeal carcinoma, and HNSCC is well studied (15, 20, 21).

HSV is a member of the Herpesviridae family and Alphaherpesvirinae subfamily. The HSV genome is 124 - 235 kb and can induce latent infection in host cells. The HSV is divided into two HSV-1 and HSV-2 types based on the glycoprotein G of the virus (22, 23). Serological assessment of HSV in oropharyngeal cancer has indicated shorter life expectancy in patients with anti-HSV IgG and tobacco smoke coexistence (24). The role of HSV as a risk factor for OSCC has been studied previously (18, 19).

2. Objectives

The aim of the current study was to evaluate the molecular epidemiology of herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in patients with head and neck cancer (HNC).

3. Methods

3.1. Patient's Selection

For this cross-sectional study, we used 90 biopsies and 66 formalin-fixed paraffin-embedded (FFPE) samples. All of the included patients had confirmed HNC cases and written informed consent was obtained from them. All of the samples were collected from hospitals affiliated with Iran University of Medical Sciences, Tehran, Iran from 2010 to 2016. The study ethics was approved by the ethics committee of Iran University of Medical Sciences under the code no: IR.IUMS.REC.1397.737.

3.2. Nucleic Acid Extraction

A 25 mg of tissue slice (2 - 3 slices of 10 microns thickness) of all samples was used for the DNA extraction. The DNA extraction was performed by QIAamp DNA FFPE Tissue Kit (Qiagen, Dusseldorf, Germany) extraction kit. All of the procedures were conducted based on the manufacturer's protocols. The quality of extracted DNA was investigated via spectrophotometry using NanoDrop ND-1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA). All of the extracted samples were stored at -70°C before the polymerase chain reaction (PCR) assessment.

3.3. HSV Detection and Typing

HSV detection was performed through the conventional polymerase chain reaction (PCR) using the following primers of forward 5'-ATGGTGAACATCGACATGTACGG-3' (25) and reverse 5'-GTAGATGGTGCGGGTGATGTT-3' with the PCR product size of 285 kb. Furthermore, the HSV typing was performed by a multiplex PCR using forward 5'-ATGGTGAACATCGACATGTACGG-3' and reverse 5'-CCTCGCGTTCGTCCTCGTCCTCC-3' For HSV-1 and forward 5'-ATGGTGAACATCGACATGTACGG-3' and reverse 5'-CCTCCTTGTCGAGGCCCCGAAAC-3' for HSV-2 with their product sizes being 469 and 391 kb, respectively (25). For the heating program, we used a Bio-Rad thermocycler (T100^{TM} Thermal Cycler) instrument. For the first round of HSV detection, we used 5 min 95°C (pre denaturation step) followed by 40 cycles of 30s at 95°C (denaturation), 30s at 55°C (annealing) and 30s at 72°C (extension), and a final extension at 72°C 5 min. The HSV multiplex PCR for typing was performed at 5 min 95°C, 40 cycles of 30 min 95°C, 30 s at 60°C, 30 s at 72°C, and one step final extension at 72°C 7 min. The PCR products were visualized and stained in 2% gel agarose electrophoresis.

3.4. Statistical Analysis

For the statistical assessment, we used SPSS version 22 (SPSS Inc., Chicago, IL, USA) with the significant results considered as P value > 0.05. The statistical assessment based on variables was performed by non-parametric Mann-Whitney U and chi-square test.

4. Results

4.1. Patients' Demographic and Clinicopathological Characteristics

A total of 156 samples including 90 (57.3%) biopsy and 66 (42%) formalin-fixed paraffin-embedded (FFPE) samples were assessed in this study. Out of all cases, 121 (77.1%) were male and 35 (22.3%) were female. The mean $\pm$ SD of the patients' age was 60.3 $\pm$ 12.65 and the mean $\pm$ SD of males and females age was 60.78 $\pm$ 11.98 and 56.74 $\pm$ 14.9, respectively. The HNC samples included larynx, tongue, glands (parotid and tonsil), nasopharynx, pharynx, vocal cord, palatine, glottis, nasal, mandibular, lip, neck, and face. More details of the patient's demographic data and tumor characteristics are summarized in Table 1. Squamous cell carcinoma (SCC) was found in 151 (96.2%) and basaloid cell carcinoma (BCC) was observed in 4 (2.5%), with adenocarcinoma seen in 1 (0.6%) patient. Also, 76 (48.4%) patients had lymph node involvement by cancer while 80 (51%) did not show any lymph node involvement ($P > 0.05$). Of all cases, 83 (52.9%) had tumor invasion to adjacent tissue, while in 70 (46.5%) cases no invasion was found ($P > 0.05$). Differentiation pattern of tumor cells showed that 47 (30.1%) were well-differentiated, 41 (26.3%) moderately, 30 (19.2%) poorly, and 38 (28.3%) un-differentiated ($P < 0.05$). The statistical assessment indicated a significant association between lymph node invasion and differentiation pattern of

tumors ($P < 0.05$). Most of the un-differentiated tumors had lymph node involvement.

4.2. HSV Infection and HSV Typing

The HSV infection and typing were assessed by PCR as mentioned above. PCR results for the HSV infection indicated that the HSV genome was present in 4 (2.6%) samples. The HSV infection did not show any statistically significant association with any other parameters. Furthermore, the typing of HSV isolates indicated that 3 (75%) of HSV infected cases were HSV-1 and 1 (25%) was HSV-2. The complete characteristics of the HSV positive cases are summarized in Table 2.

5. Discussion

The aim of the current study was to evaluate the molecular epidemiology of HSV-1 and HSV-2 in patients with head and neck cancer (HNC). The mean ± SD of the patients' age was 60.3 ± 12.65, and 121 (77.1%) were male. Tumor location included larynx 35 (22.4%), tongue 29 (18.6%), glands (parotid and tonsil) 6 (3.8%), nasopharynx 12 (7.6%), pharynx 9 (5.7%), vocal cord 33 (21%), palatine 3 (1.9%), glottis 17 (10.8%), nasal 2 (1.3%), mandibular 4 (2.5%), lip 4 (2.5%), neck 1 (0.6%), and face 1 (0.6%). Using the PCR, HSV genome was found in 4 (2.6%) samples, of which 3 (75%) were HSV-1.

HNSCC malignancy is associated with the cancer location, gender, and geographical region. Its incidence ranges are from 5% to 50% of all malignancies in different countries. Sothern Asia and Europe are the most prevalent geographical locations (7, 26). Also, HNSCC seems to be more common in the male gender (7, 27). The most prevalent HNSCC in India is oral and tongue cancer while in Hong Kong is nasopharynx cancer (28, 29). The prevalence of the HSV in HNSCC different types including OSCC and pharyngolaryngeal squamous carcinoma ranges within 5% - 25% (19, 30). In the study conducted by Larsson et al. (31), the association of HSV infection and HNSCC outcome was investigated. They reported that HSV prevalence had no significant differences. Also, the importance of the IgM antibody against HSV in oral malignancies was suggested by Correia et al. (32). The anti-HSV Ab levels could act as a prognostic factor in HNSCC progression (31). The current study aimed to assess molecular epidemiology of HSV as a preliminary study in Iranian patients with HNC and further studies should evaluate the prognostic factors. Although we found the lowest rate of HSV infection in patients with HNC, it could be due to our specific population, limited sample size, or geographical and racial differences. Nevertheless, our findings showed the same pattern of HNC prevalence in the male gender as with previous studies (7, 27).

Parker et al. (33) indicated that HSV and HPV infections are both play an important role in HNSCC incidence. In a study by Osman and et al. (34), there was a significant association between HSV infection and mandibular OSCC. Also, they reported that HSV-1 and HSV-2 prevalence in HNSCC was 18% and 6%, respectively. Based on the current study results, there were no HSV positive cases with mandibular tumors. This might be due to the differences in the patients' groups or the sample size. Regardless of the differences between our study results and other studies, we found the majority of HSV-1 isolates infected our patients with HNC. The authors of previous study on HNC patients showed that HPV was found in 3.2% of the patients (35). According to the results of the present study which found 2.6% of the patients were infected by HSV genome, it could be concluded that the rate of these viral infections is low.

In the study by Jalouli et al. (18), the prevalence of HSV in patients with OSCC was calculated 15% worldwide, while the highest HSV prevalence was seen in the UK (55%). These results clearly suggested the geographical differences in the HSV prevalence in patients with OSCC which could magnify the epidemiological importance of the HSV infection in this group.

Mokhtari et al. (36) reported the HSV-1 in 5% of patients with OSCC. Also, Bashir et al. (37) reported a higher prevalence of HSV-2 in OSCC Paraffin-embedded well-differentiated tissue samples. The prevalence of the HSV-1 and HSV-2 in Bashir's study was 7.5% and 15%, respectively. Also, 5% of the investigated samples were co-infected by HSV-1 and HSV-2. Our results are likely similar to the study of Mokhtari et al. (36), which confirmed the low rate of HSV infection in HNC patient's tumor tissue.

Further, based on the current study results, 121 (77.1%) of HNSCC patients were male. This domination of male gender has been mentioned in an earlier study (38). In a study of Rautava et al. (39) they were focused on HPV detection and HPV typing in HNSCC patients and also, they identified HSV-1 co-infection in 6.6% of cases with HPV infection. In Rautava et al.'s study (39), the HNSCC tumors included lip 5, oral cavity 37, oropharynx 31, nasopharynx 8, hypopharynx 15, and larynx 10 cases. Although we used different cancer locations, they did not address the exact HSV positive locations.

Devilleres-Mendoza and Chang (40) discussed a case with laryngeal squamous cell carcinoma with HSV associated cytopathology features. In our study, we could detect the HSV genome in the larynx of the case. This result might be a clue for further investigations. Nevertheless, in the study conducted by Furukawa et al. (41), it was suggested that the HSV-1 strain RH-2 (where the γ34.5 gene is removed from it) could be a great autophagy inducer and has potential to act as a target for viral therapy in HNSCC subjects.

Table 1. Tumor Location and Patient's Data About Gender, Lymph Node Involvement, and Differentiation Level of Tumor, Invasion and Type of Sampling[a]

HNC Anatomic Location	Total Number	Gender		Lymph Node Involvement by Tumor		Tumor Differentiation			Tumor Invasion			Sample Type	
		Male	Female	Involved	Not-Involved	Well	Moderate	Poor	Un-Differentiated	Invades	Not-Invades	Biopsy	FFPE
Larynx	35 (22.4)	27 (22.3)	8 (22.9)	19 (25)	16 (20)	8 (17)	9 (22)	13 (37.1)	8 (29)	24 (28.9)	11 (15.1)	17 (18.9)	18 (27.3)
Tongue	29 (18.6)	16 (13.2)	13 (37.1)	11 (14.5)	18 (22.5)	17 (36.2)	6 (14.6)	4 (13.3)	2 (7.4)	12 (14.5)	17 (23.3)	11 (12.2)	18 (27.3)
Glands (parotid and tonsil)	6 (3.8)	4 (3.3)	2 (5.7)	2 (2.6)	4 (5)	2 (4.3)	2 (4.9)	1 (3.3)	1 (3.7)	3 (3.6)	3 (4.1)	2 (2.2)	4 (6.1)
Nasopharynx	12 (7.6)	10 (8.3)	2 (5.7)	7 (9.2)	5 (6.3)	0	1 (2.4)	1 (3.3)	0	3 (3.6)	9 (12.3)	9 (10)	3 (4.5)
Pharynx	9 (5.7)	5 (4.1)	4 (11.4)	6 (7.9%)	3 (3.8)	2 (4.3)	3 (7.3)	0	4 (14.8)	4 (4.8)	5 (6.8)	8 (8.9)	1 (1.5)
Vocal cord	33 (21)	33 (27.3)	0 (0)	18 (23.7)	15 (18.8)	10 (21.3)	11 (26.8)	6 (20)	6 (22.2)	17 (20.5)	16 (21.9)	30 (33.3)	3 (4.5)
Palatine	3 (1.9)	2 (1.7)	1 (2.9)	0	3 (3.8)	1 (2.1)	0	1 (3.3)	0	2 (2.4)	1 (1.4)	1 (1.1)	2 (3)
Glottis	17 (10.8)	15 (12.4)	2 (5.7)	10 (13.2)	7 (8.8)	2 (4.3)	6 (14.6)	0	3 (11.)	10 (12)	7 (9.6)	9 (10)	8 (12.1)
Nasal	2 (1.3)	1 (0.8)	1 (2.9)	2 (2.6)	0	1 (1.2)	0	0	1 (3.7)	2 (2.4)	0	1 (1.1)	1 (1.5)
Mandibular	4 (2.5)	3 (2.5)	1 (2.9)	0	4 (5)	2 (4.3)	1 (2.4)	0	0	3 (3.6)	1 (1.4)	0	4 (6.1)
Lip	4 (2.5)	3 (2.5)	1 (2.9)	1 (1.3)	3 (3.8)	1 (1.2)	2 (4.9)	0	1 (3.7)	2 (2.4)	2 (2.7)	2 (2.2)	2 (3)
Neck	1 (0.6)	1 (0.8)	0 (0)	0	1 (1.3)	0	0	0	1 (3.7)	1 (1.2)	0	0	1 (1.5)
Face	1 (0.6)	1 (0.8)	0 (0)	0	1 (1.3)	1 (1.2)	0	0	0	0	1 (1.4)	0	1 (1.5)
P value[b]	-	P< 0.05[c]		P> 0.05		P< 0.05[c]			P> 0.05			P> 0.05	

Abbreviation: FFPE, Formalin Fixed Paraffin Embedded tissue; HNC, head and neck cancers.
[a] Values are expressed as No. (%).
[b] P value by chi-square for assessing the tumor location with other parameters in columns.
[c] Not significant.

Table 2. Demographic and Clinicopathological Characteristics of HSV Positive Cases

Case number	HSV Type	Sampling Type	Gender	Age, y	Tumor Location	Tumor Type	Lymph Node Involvement	Tumor Invasion
1	HSV-1	Biopsy	Female	65	Pharynx	SCC	Yes	Yes
2	HSV-1	FFPE	Female	68	Larynx	BCC	Yes	Yes
3	HSV-1	FFPE	Male	54	Palatine	SCC	No	No
4	HSV-2	Biopsy	Male	58	Tongue	SCC	Yes	No

Abbreviations: BCC, basaloid cell carcinoma; FFPE, Formalin Fixed Paraffin Embedded tissue; HSV, herpes Simplex virus; SCC, Squamous cell carcinoma.

In conclusion, the current study was done for the first time in Iranian patients with HNC with the results indicating that the HSV prevalence in HNC patients is 2.6% and HSV-1 is the dominant type (75%). The HSV was present in different anatomical locations including pharynx, larynx, palatine, and tongue. This finding highlighted the importance of further investigation of the exact role of the HSV in HNC.

Footnotes

Authors' Contribution: Study concept and design: MHKN and AD. Analysis and interpretation of data: AT and ASJ. Drafting of the manuscript: HK, MP. Critical revision of the manuscript for important intellectual content: FST, FZ, and ST. Statistical analysis: MHKN and AT.

Conflict of Interests: None to declare by all authors.

Ethical Approval: The ethical approval code was IR.IUMS.REC.1397.737.

Funding/Support: This study was done by the supported grant of Iran University of Medical Sciences, Tehran, Iran (code no.: 97-275-12462).

Informed Consent: All of the included patients had confirmed HNC cases and written informed consent was obtained from them.

References

1. 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. doi: 10.7314/APJCP.2014.15.17.7029. [PubMed: 25227786].
2. Mirzaei M, Hosseini S, Ghoncheh M, Soheilipour F, Soltani S, Soheilipour F, et al. Epidemiology and trend of head and neck cancers in Iran. *Global journal of health science*. 2016;**8**(1):189. doi: 10.5539/gjhs.v8n1p189. [PubMed: 26234980].
3. Tabatabaie F, Tafreshi ZA, Shahmohammad N, Pirestani M. Molecular detection of microsporidiosis in various samples of Iranian immunocompromised patients. *Journal of Parasitic Diseases*. 2015;**39**(4):634–8. doi: 10.1007/s12639-014-0432-8. [PubMed: 26688625].

4. Warnakulasuriya S. Global epidemiology of oral and oropharyngeal cancer. *Oral oncology*. 2009;**45**(4-5):309–16. doi: 10.1016/j.oraloncology.2008.06.002. [PubMed: 18804401].
5. Mafi N, Kadivar M, Hosseini N, Ahmadi S, Zare-Mirzaie A. Head and neck squamous cell carcinoma in Iranian patients and risk factors in young adults: a fifteen-year study. *Asian Pacific Journal of Cancer Prevention*. 2012;**13**(7):3373–8. doi: 10.7314/APJCP.2012.13.7.3373. [PubMed: 22994763].
6. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA oncology*. 2017;**3**(4):524–48. doi: 10.1001/jamaoncol.2016.5688. [PubMed: 27918777].
7. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. *CA: a cancer journal for clinicians*. 2016;**66**(1):7–30. doi: 10.3322/caac.21332. [PubMed: 26742998].
8. Gerami H, Kousha AR, Aghajanpour SM, Kazem Nejad E. A Report of Relative Frequency of Head and Neck Tumors and Respective Factors in Guilan Province. *Journal of Guilan University of Medical Sciences*. 2015;**24**(93):1–9.
9. Chen YK, Huang HC, Lin LM, Lin CC. Primary oral squamous cell carcinoma: an analysis of 703 cases in southern Taiwan. *Oral oncology*. 1999;**35**(2):173–9. doi: 10.1016/S1368-8375(98)00101-8.
10. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA: a cancer journal for clinicians*. 2005;**55**(2):74–108. doi: 10.3322/canjclin.55.2.74. [PubMed: 15761078].
11. Rodriguez T, Altieri A, Chatenoud L, Gallus S, Bosetti C, Negri E, et al. Risk factors for oral and pharyngeal cancer in young adults. *Oral oncology*. 2004;**40**(2):207–13. doi: 10.1016/j.oraloncology.2003.08.014. [PubMed: 14693246].
12. Wyss A, Hashibe M, Chuang S, Lee YA, Zhang Z, Yu G, et al. Cigarette, cigar, and pipe smoking and the risk of head and neck cancers: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *American journal of epidemiology*. 2013;**178**(5):679–90. doi: 10.1093/aje/kwt029. [PubMed: 23817919].
13. Wyss AB, Hashibe M, Lee YA, Chuang S, Muscat J, Chen C, et al. Smokeless tobacco use and the risk of head and neck cancer: pooled analysis of US studies in the INHANCE consortium. *American journal of epidemiology*. 2016:1–14.
14. Rettig EM, D'Souza G. Epidemiology of head and neck cancer. *Surgical Oncology Clinics*. 2015;**24**(3):379–96. [PubMed: 25979389].
15. Metgud R, Astekar M, Verma M, Sharma A. Role of viruses in oral squamous cell carcinoma. *Oncology reviews*. 2012;**6**(2). doi: 10.4081/oncol.2012.e21. [PubMed: 25992219].
16. Kansy K, Thiele O, Freier K. The role of human papillomavirus in oral squamous cell carcinoma: myth and reality. *Oral and maxillofacial surgery*. 2014;**18**(2):165–72. doi: 10.1007/s10006-012-0383-0. [PubMed: 23242943].
17. Webster-Cyriaque J, Edwards RH, Quinlivan EB, Patton L, Wohl D, Raab-Traub N. Epstein-Barr virus and human herpesvirus 8 prevalence in human immunodeficiency virus-associated oral mucosal lesions. *The Journal of infectious diseases*. 1997;**175**(6):1324–32. doi: 10.1086/516463. [PubMed: 9180170].
18. Jalouli J, Ibrahim SO, Mehrotra R, Jalouli MM, Sapkota D, Larsson P, et al. Prevalence of viral (HPV, EBV, HSV) infections in oral submucous fibrosis and oral cancer from India. *Acta otolaryngologica*. 2010;**130**(11):1306–11. doi: 10.3109/00016481003782041. [PubMed: 20441534].
19. Jalouli J, Jalouli MM, Sapkota D, Ibrahim SO, Larsson P, Sand L. Human papilloma virus, herpes simplex virus and epstein barr virus in oral squamous cell carcinoma from eight different countries. *Anticancer research*. 2012;**32**(2):571–80.
20. Mirzamani N, Salehian P, Farhadi M. Detection of EBV and HPV in nasopharyngeal carcinoma by in situ hybridization. *Experimental and molecular pathology*. 2006;**81**(3):231–4. doi: 10.1016/j.yexmp.2006.04.006. [PubMed: 16787643].
21. Deng Z, Uehara T, Maeda H, Hasegawa M, Matayoshi S, Kiyuna A, et al. Epstein-Barr virus and human papillomavirus infections and genotype distribution in head and neck cancers. *PloS one*. 2014;**9**(11). e113702. doi: 10.1371/journal.pone.0113702. [PubMed: 25405488].
22. Taylor GS, Long HM, Brooks JM, Rickinson AB, Hislop AD. The immunology of Epstein-Barr virus-induced disease. *Annual review of immunology*. 2015;**33**:787–821. doi: 10.1146/annurev-immunol-032414-112326. [PubMed: 25706097].
23. Turunen A, Hukkanen V, Nygårdas M, Kulmala J, Syrjänen S. The combined effects of irradiation and herpes simplex virus type 1 infection on an immortal gingival cell line. *Virology journal*. 2014;**11**(1):1. doi: 10.1186/1743-422X-11-125. [PubMed: 25005804].
24. Starr JR, Daling JR, Fitzgibbons ED, Madeleine MM, Ashley R, Galloway DA, et al. Serologic evidence of herpes simplex virus 1 infection and oropharyngeal cancer risk. *Cancer research*. 2001;**61**(23):8459–64.
25. Madhavan HN, Priya K, Bagyalakshmi R. Phenotypic and genotypic methods for the detection of herpes simplex virus serotypes. *Journal of virological methods*. 2003;**108**(1):97–102. doi: 10.1016/S0166-0934(02)00264-1.
26. Gatta G, Botta L, Sánchez MJ, Anderson LA, Pierannunzio D, Licitra L, et al. Prognoses and improvement for head and neck cancers diagnosed in Europe in early 2000s: The EUROCARE-5 population-based study. *European journal of cancer*. 2015;**51**(15):2130–43. doi: 10.1016/j.ejca.2015.07.043. [PubMed: 26421817].
27. Reddy RB, Bhat AR, James BL, Govindan SV, Mathew R, Ravindra DR, et al. Meta-Analyses of Microarray Datasets Identifies ANO1 and FADD as Prognostic Markers of Head and Neck Cancer. *PloS one*. 2016;**11**(1). e0147409. doi: 10.1371/journal.pone.0147409. [PubMed: 26808319].
28. Bray F, Ren J, Masuyer E, Ferlay J. Global estimates of cancer prevalence for 27 sites in the adult population in 2008. *International journal of cancer*. 2013;**132**(5):1133–45. doi: 10.1002/ijc.27711. [PubMed: 22752881].
29. Lambert R, Sauvaget C, de Camargo Cancela M, Sankaranarayanan R. Epidemiology of cancer from the oral cavity and oropharynx. *European journal of gastroenterology & hepatology*. 2011;**23**(8):633–41. doi: 10.1097/MEG.0b013e3283484795. [PubMed: 21654320].
30. Zheng Y, Xia P, Zheng H, Takahashi H, Masuda S, Takano Y. The screening of viral risk factors in tongue and pharyngolaryngeal squamous carcinoma. *Anticancer research*. 2010;**30**(4):1233–8.
31. Larsson P-A, Edström S, Westin T, Nordkvist A, Hirsch JM, Vahlne A. Reactivity against herpes simplex virus in patients with head and neck cancer. *International journal of cancer*. 1991;**49**(1):14–8. doi: 10.1002/ijc.2910490104. [PubMed: 1651906].
32. Correia AV, Coelho MR, de Oliveira Mendes Cahu GG, de Almeida Silva JL, da Mota Vasconcelos Brasil C, de Castro JF. Seroprevalence of HSV-1/2 and correlation with aggravation of oral mucositis in patients with squamous cell carcinoma of the head and neck region submitted to antineoplastic treatment. *Support Care Cancer*. 2015;**23**(7):2105–11. doi: 10.1007/s00520-014-2558-8. [PubMed: 25547479].
33. Parker TM, Smith EM, Ritchie JM, Haugen TH, Vonka V, Turek LP, et al. Head and neck cancer associated with herpes simplex virus 1 and 2 and other risk factors. *Oral oncology*. 2006;**42**(3):288–96. doi: 10.1016/j.oraloncology.2005.08.003. [PubMed: 16324878].
34. Osman SA, Enan KA, Mohamed EA. Molecular detection of herpes simplex virus (1, 2) in oral squamous cell carcinoma at Khartoum. *Clinical Medicine Journal*. 2017;**2**:10–4.
35. Niya MHK, Tameshkel FS, Panahi M, Salim FB, Monavari SHR, Keyvani H. Human papillomavirus investigation in head and neck squamous cell carcinoma: initial report from the low risk HPV types associations. *Asian Pacific journal of cancer prevention: APJCP*. 2017;**18**(9):2573.
36. Mokhtari M, Beiraghdar M. Evaluation of the prevalence of herpes simplex-1 infection in oral squamous cell carcinoma specimens in Alzahra and Kashani Hospitals with polymerase chain reaction method in 2012-2013. *Advanced biomedical research*. 2015;**4**.
37. Bashir RA, Elhag WI. Molecular Detection of Herpes Simplex Virus

Types [1 and 2] in Oropharyngeal Squamous Cell Carcinoma (OSCC) in Khartoum Dental Education Hospital. *Journal of Advances in Medicine and Medical Research*. 2018:1–6. doi: 10.9734/JAMMR/2018/41717.

38. Stenson KM, Brockstein BE, Ross ME. Epidemiology and risk factors for head and neck cancer. *UpToDate*. 2016.
39. Rautava J, Kuuskoski J, Syrjanen K, Grenman R, Syrjanen S. HPV genotypes and their prognostic significance in head and neck squamous cell carcinomas. *J Clin Virol*. 2012;**53**(2):116–20. doi: 10.1016/j.jcv.2011.11.005. [PubMed: 22177275].
40. Devilleres-Mendoza DD, Chang JV. Cytopathologic Herpes Simplex Virus Features In Laryngeal Squamous Cell Carcinoma. *PhiliPPine Journal of otolaryngology-head and neck Surgery*. 2016;**31**(1):61–4. doi: 10.32412/pjohns.v31i1.325.
41. Furukawa Y, Takasu A, Yura Y. Role of autophagy in oncolytic herpes simplex virus type 1-induced cell death in squamous cell carcinoma cells. *Cancer gene therapy*. 2017;**24**(9):393. doi: 10.1038/cgt.2017.33. [PubMed: 28984290].