Journal of Advances in Medicine and Medical Research

26(9): 1-6, 2018; Article no.JAMMR.41717
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Molecular Detection of Herpes Simplex Virus Types [1 and 2] in Oropharyngeal Squamous Cell Carcinoma (OSCC) in Khartoum Dental Education Hospital

Razan Abdalla Bashir[1*] and Wafa Ibrahim Elhag[2]

[1]Department of Microbiology, Faculty of Medical Laboratory Sciences, Al-Neelain University, Sudan.
[2]Department of Medical Microbiology, Al-Neelain University, Sudan.

Authors' contributions

This work was carried out in collaboration between both authors. Author RAB designed the study, wrote the protocol, wrote the manuscript and managed the literature searches. Author WIE managed the analyses of the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/41717
Editor(s):
(1) Emmanouil (Manolis) Magiorkinis, General Hospital of Rethumnon, Trantalidou 19-21, Rethymnon 74100, Greece.
Reviewers:
(1) Shamala Moodley, Mangosuthu University of Technology, South Africa.
(2) Renshan Sun, Third Military Medical University, China.
Complete Peer review History: http://www.sciencedomain.org/review-history/24989

Original Research Article

Received 2nd March 2018
Accepted 13th May 2018
Published 5th June 2018

ABSTRACT

Background: Oral Squamous cell carcinoma (OSCC) is the most frequent oral cavity malignancy accounting for over 90% of oral cancers. The aim of this study was to detect of Herpes simplex virus types [1 and 2] in Oral Squamous Cell Carcinoma (OSCC).
Methodology: Paraffin embedded well differentiated oropharyngeal squamous cell carcinoma tissues and benign lesions from 50 patients attending the Cancer Department in Khartoum Dental Education Hospital during 2015-2016, were examined for the presence of HSV-1 and HSV-2 genes using polymerase chain reaction (PCR).
Ethical approval was obtained from Al Neelain University research ethical bord and informed consent was taken from patients after full explanation of the purpose of the study.
Results: Out of a total 50 samples: among 40 OSCC paraffin-embedded tissues specimens, HSV-1 and HSV-2 were identified in 3 (7.5%) and 6 (15%) of samples respectively. While 2 (5%)

**Corresponding author: E-mail: razanabdullahhamed93@gmail.com;*

samples were co-infected with HSV type 1 and 2. Among 10 benign tumors specimens 1 (10%) sample was positive for both HSV types 1 and 2 (co-infection).
Conclusion: This study showed a higher prevalence of HSV-2 compared with HSV-1. Further studies are needed to investigate relationship between HSV-2 and behavioral factors (smoking, tobacco and alcohol use, or change of sexual behaviors) in developing of OSCC.

Keywords: Herpes simplex virus; oropharyngeal squamous cell carcinoma; PCR; paraffin-embedded tissues.

1. INTRODUCTION

Oral cancer has been defined as "a neoplasm involving the oral cavity which begins at the lip and ends at the anterior pillar of the throat" [1]. Oral Squamous cell carcinoma (OSCC) is the most frequent oral cavity malignancy accounting for over 90% of oral cancers [2]. It represents the sixth most frequent malignant tumor worldwide [2].

OSCC was the commonest malignant lesions in Sudan representing 66.5% and its prevalence was higher in men than in women [3].

The etiology of OSCC is considered to be multifactorial and the factors influencing it include environmental factors, lifestyle, infectious agents and genetic alterations. The main predisposing factors are tobacco and alcohol abuse [4]. Oncogenic viruses played an important role in the etiology of OSCC [5]. The most commonly viruses implicated are the human papilloma virus (HPV) [6,7], herpes group viruses [8], adenoviruses [9] and *hepatitis C viruses* [10,11].

Herpes simplex belongs to a group of eight related viruses, including (HSV-1) and (HSV-2), *varicella zoster virus, Epstein-Barr virus, and cytomegalovirus* [12]. HSV-1 is an important pathogen that causes a variety of clinical manifestations in humans, predominantly infects the oral mucosa and causes oral "cold" sores. It has the ability to remain latent in host neurons for life, and can reactivate to cause lesions at or near the site of initial infection. HSV-2 antibodies are considered to reflect genital infection and can be transmitted if a person infected with genital herpes receives oral sex, causing oral herpes in their partner [13,14,15,16,17,18].

The role of HSV in the development of OSCC has been least investigated. However, animal study has shown the relation of HSV infection and simulated snuff that enhance the development of micro-invasive squamous cell carcinoma in hamster buccal [19] and other animal study [20]. Furthermore, clinical studies [21], has also shown a possible interaction between the use of tobacco and HSV-1 in the development of OSCC.

A combination of HSV seropositivity and a history of cigarette smoking is associated with a higher risk of oral cancer has been reported [22]. The role of *herpes simplex viruses*, HSV-1 and HSV-2, as co-factors in association with tobacco, alcohol, or HPV-16 infection has also been examined, HPV-16 infection was associated with an increased risk of OSCC, in addition to age, tobacco, alcohol use, and number of sexual partners was the most important [23].

Early studies attempted to verify whether DNA of HSV could be detected in oral cancers, and preliminary reports did indicate the presence of both/either viral DNA and/or RNA [24].

Recently studies suggested the possibility that HSV transforms cells by acting as a mutagen. Studies of the frequency of mutations at the hypoxanthine guanine ribosyl transferase locus of mammalian cell genomic DNA showed an increase in mutation frequency of up to 2-4 fold following infection by HSV 1. These had been inactivated by UV light [25], The same phenomenon has been observed by Pilon et al. in cells infected by HSV-2 [26].

In a review study from 2010, Meurman claimed that HSV infections link statistically with oral carcinogenesis and that antibody levels to HSV-1 and HSV-2 are increased in OSCC patients [27].

In a population-based study from the USA, the authors concluded that HSV-1 may enhance the development of OSCC [28], and in another study, HSV-1 antibody was associated with a slightly increased risk of head and neck cancer [29].

Furthermore, in a study in 1983, over 50% of RNA complementary to HSV-1 and HSV-2 were found in biopsy specimens from patients with

OSCC, the specimens were examined by in *situ hybridization* [30].

This study aimed to determine the role of HSV-1 and HSV-2 in OSCC by detection the virus DNA in paraffin embedded tissues specimens.

2. MATERIALS AND METHODS

2.1 Study Type Design

This is a hospital based descriptive Cross Sectional study conducted at Khartoum Dental Education Hospital including Oropharyngeal squamous cell carcinoma patients.

Ethical approval was obtained from the Al Neelain University and informed consent was taken from patients after full explanation of the purpose of the study, data was collected using structural questionnaire.

2.2 Sample Collection

Paraffin embedded well differentiated oropharyngial squamous cell carcinoma tissues and benign tumors were collected from Cancer patients at the Departments of cancer in Khartoum Dental Education Hospital. In all 50 samples of which 40 patients diagnosed with well differentiated oropharyngeal squamous cell carcinoma, and 10 patients diagnosed with benign oral tumors were enrolled in the study.

2.3 DNA Extraction

The QIAamp (QIAGEN - Germany) kit use for purification of DNA from formalin fixed paraffin embedded tissues. In brief xylene was used to removed paraffin, then the tissues were rehydrated using a series of ethanol Washes. Proteinase K was then added to lyse the cells and release the DNA. After that, the lysate applied to the QIAamp MinElute column and residual contaminants (proteins and enzymes) are washed away by buffers. The DNA collected in membrane was eluted in buffer or water and storaged in -20°C until used.

2.4 Polymarase Chain Reaction (PCR)

PCR was performed by processing the extracted DNA with primers (Macrogen – Korea) that are specific for HSV-1 and HSV-2 genes. The primers used for HSV1 were forward:

5'-GTTAGGGAGTTGTTCGGTCATAAGCT-3'
Reverse: 5'-TCGGCCATCTTGAGCATCC-3', the HSV2 primers were: Forward: 5'-GTCGGTGTGGTGTTCGGTCATAAGCT-3'
Reverse: 5'-GCTGAATCTGGTAAACACGCTTC-3'.

Amplification was as follows:

Initial denaturation at 94°C for seven minutes, denaturation at 94°C for one minute, annealing at 56°C for two minutes and extension at 75°C for one minute and half, and final extension at 75°C for seven minutes.

2.5 Gel Electrophoresis

PCR products were detected in 2% agarose gel stained with ethidium bromide and compared with 100 bp DNA ladder. The band length of HSV-1 and 2 was amplicons were 208 bp and 267 bp respectively.

3. RESULTS

Out of a total fifty paraffin-embedded tissues, 40 (80%) were OSCC specimens and 10 (20%) were benign tumor specimens.

This study population age ranged between 13 to 90 with mean age of 51.5 years.

Among 40 OSCC paraffin-embedded tissues specimens, HSV-1 and HSV-2 were identified in 3 (7.5%) and 6 (15%) of samples respectively. While 2 (5%) samples were co-infected with HSV type 1 and 2, however among 10 benign tumors specimens Only 1 (10%) sample was positive for HSV types 1 and 2 (co-infection) Table 1.

Thirty (60%) of total population were males (6 patients with benign tumors and 24 with OSCC), and 20 (40%) were females (4 patients with benign tumors and 16 with OSCC). High positivity was observed among males (7 (58.3%) and 5 (41.7%) were females) for HSV-1 or HSV-2 or co-infected with both viruses.

Among 12 positive patients, HSV-1 positive were belonged to age range from 60 to 90, while for HSV-2 belonged to ranged from 40 to 70.

Anatomical sites of OSCC were mainly found in tongue, mandible, gum, and lips (22.5%, 20%, 12.5%, and 12.5% respectively), and less frequently found in jaw, labia, palate and salivary gland (2.5%, 5%, 5%, 5% respectively). while benign tumors were mainly found in mandible (30%).

Table 1. PCR results for HSV-1, HSV-2, and co-infecrion among study population

	No %	HSV1		HSV2		HSV1,2	
		+ve	-ve	+ve	-ve	+ve	-ve
OSCC	40 (80.0%)	3 (7.5%)	37(92.5%)	6(15.0%)	34(85.0%)	2(5.0%)	38(95.0%)
Benign tumor	10 (20.0%)	0	10(100%)	0	10 (100%)	1(10.0%)	9(90.0%)
Total	50 (100%)	3 (6.0%)	47(94.0%)	6(12.0%)	44(88.0%)	3 (6.0%)	47(94.0%)

Table 2. Anatomical sites of positive lesions for HSV-1, HSV-2 and co-infection among study population

Site	SV1+ve		HSV-2 +ve		HSV1 and2	
	OSCC	Benign tumors	OSCC	Benign tumors	OSCC	Benign tumors
Tongue	1	-	3	-	-	-
Mandible	1	-	-	-	1	-
Lip	-	-	-	-	1	-
Jaw	-	-	1	-	-	1
Buccal mucosa	-	-	1	-	-	-
Palate	1	-	1	-	-	-
Labia	-	-	-	-	-	-
Maxilla	-	-	-	-	-	-
Gum	-	-	-	-	-	-
Slivery gland	-	-	-	-	-	-
Total:	3(7.5%)	0(0%)	6(15%)	0(0%)	2(5%)	1(10%)

In OSCC positive PCR samples, HSV-1 most found in mandibles (18%), while HSV-2 most found in tongues (27%). While the 1 positive sample of benign tumor was co-infected by two viruses found in jaw (Table 2).

4. DISCUSSION

Oral cancer is an important cause of morbidity and mortality, especially in developing countries, and its prevalence may rise in the foreseeable future [31]. The studies of virus-associated head and neck cancers have provided many new insights into key mechanisms of carcinogenesis. The human tumor virus oncogenes play central roles in viral life cycles and their oncogenic potential is a manifestation of these activities [31].

Of all 50 samples, 40 were OSCC paraffin-embedded tissues specimens, HSV-1 and HSV-2 were identified in 3 (7.5%) and 6 (15%) of samples respectively. While 2 (5%) samples were co-infected with HSV type 1 and 2. In 10 benign tumors specimens Only 1 (10%) sample was positive for HSV types 1 and 2 (co-infection).

When compared with previous research finding it found to be similar to result of Jalouli et al. 2015. Iran who investigated the prevalence of HPV, HSV, and EBV DNA by PCR sequencing in brush biopsies obtained from patients with oral dysplasia and OSCC. Their findings illustrated that prevalence of HSV, HPV and EBV infections were common and may influence oral health and cancer development [32].

Also, similar study was aimed to detect the presence of HSV-1 DNA and HSV-2 DNA was previously investigated in OSCC patients attending Khartoum Teaching Dental Hospital by Osman et al. [33]. The prevalence of HSV-1 positive samples was 18.6%, and HSV-2 was 6.8% [33]. The authors speculated that HSV-1 has an important role in OSCC [33]. In contrast to our results that showed 7.5% and 15% prevalence for HSV-1 and HSV-2 respectively, and suggesting that HSV-2 may be more associated with OSCC development than HSV-1. The reason for this discrepancy is difficult to explain and may be related to small sample size used, and may need for further larger studies to be carried out.

5. CONCLUSION

This study showed higher prevalence of HSV-2 compared with HSV-1 among OSCC patients.

Further studies are needed to investigate relation between HSV-2 and behavioral factors (smoking, tobacco and alcohol use, or change of sexual behaviors) in developing of OSCC.

CONSENT

As per international standard or university standard, patient's written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Mathur PT, Dayal PK, Pai KM. Correlation of clinical patterns of oral squamous cell carcinoma with age, site, sex and habits. Journal of Indian Academy of Oral Medicine and Radiology. 2011;81–85.
2. Kulkarni PR, Rani H, Vimalambike MG, Ravishankar S. Opportunistic screening for cervical cancer in a Tertiary Hospital in Karnataka, India. Asian Pacific Journal of Cancer Prevention. 2013;5101–5105.
3. Idris AM, Ahmed HM, Mukhtar BI, Gadir AF, et al. Descriptive epidemiology of oral neoplasms in Sudan 1970-1985 and the role of Toombak. International Journal of Cancer. 1995;61(2):155-158.
4. Garbuglia Ar. Human papillomavirus in head and neck cancer. Cancers. 2014;6: 1705–1726.
5. Alibek K, Kakpenova A, Yeldar Baiken Y. Role of infectious agents in the carcinogenesis of brain and head and neck cancers. Infect Agent Cancer. 2013;8:7–16.
6. Gondivkar SM, Parikh RV, Gadbail AR, et al. Involvement of viral factors with head and neck cancers. Oral Oncology. 2012;48(3):195–199.
7. Greer Jr. RO, Douglas Jr. JM, Breese P, Crosby LK. Evaluation of oral and laryngeal specimens for human papillomavirus (HPV) DNA by dot blots hybridization. Journal of Oral Pathology and Medicine. 1990;19(1):35–38.
8. Greer RO, Shroyer K, Crosby L. Identification of human papillomavirus DNA in smokeless tobacco keratosis and premalignant and malignant oral lesions by PCR amplification with consensus sequence primers. NIH Publication, US Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, Md, USA. 1992;92:183-190.
9. Park NH, Byung MM, Sheng LL. Role of viruses in oral carcinogenesis. NIH Publication, US Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, Md, USA. 1992;92:202-215.
10. Johnson NW. Risk markers for oral disease, oral cancer detection of patients and lesions at risk. Cambridge University Press, Cambridge, UK; 1991.
11. Nagao Y, Sata M, Tanikawa K, Itoh K, et al. High prevalence of hepatitis C virus antibody and RNA in patients with oral cancer. Journal of Oral Pathology and Medicine. 1995;24(8):354–360.
12. Wagner EK, Sandri-Goldin RM. Herpes simplex viruses: Molecular biology. In: Mahy, Regenmartel V, H. V. M, editors. Desk Encyclopedia of General Virology: Academic Press. 2008;460-468.
13. White DO, Fenner F. Medical virology, 4th ed. San Diego: Academic Press, Inc. 1994;152–153,317–347.
14. Nathanson N. Viral pathogenesis. Philadelphia: Lippincott- Raven. 1997;143–161,181–205.
15. Jerome KR, Tait JF, Koelle DM, Corey L. Herpes simplex virus type 1 renders infected cells resistant to cytotoxic T-lymphocyte induced apoptosis. Journal Virology. 1998;72:436-441.
16. Johnson DC, Hill AB. Herpes virus evasion of the immune system. Current Topic in Microbiology and Immunology. 1998;232: 149-177.
17. Scott DA, Coulter WA, Lamey PJ. Oral shedding of herpes simplex virus type 1: A review. Journal of Oral Pathology. Med. 1997;26:441-447.
18. Steiner I. Human herpes viruses latent infection in the nervous system. Immunology Review Journal. 1996;152: 157-173.
19. Stich JE, Li KK, Chun YS, et al. Effect of smokeless tobacco on the replication of

herpes simplex virus *in vitro* and on production of viral lesions in hamster cheek pouch. Arch Oral Biology. 1987;32: 291-296.

20. Larsson PA, Johansson SL, Vahlne A, Hirsch JM. Snuff tumorigenesis: Effects of long-term snuff administration after initiation with 4-nitroquinoline-*N*-oxide and herpes simplex virus type 1. Journal of Oral Pathology and Medicine. 1989;18: 187-192.
21. Yang YY, Koh LW, Tsai JH, Tsai CH, et al. Involvement of viral and chemical factors with oral cancer in Taiwan. Jpn J Clin Oncol. 2004;34:176-183.
22. Starr JR, Daling JR, Fitzgibbons ED, et al. Serologic evidence of herpes simplex virus 1 infection and oropharyngeal cancer risk. Cancer Research. 2001;23:8459–8464.
23. Parker TM, Smith EM, Ritchie JM, et al. Head and neck cancer associated with herpes simplex virus 1 and 2 and other risk factors. Oral Oncology Journal. 2006;42: 288-296.
24. Cox M, Maitland N, Scully C, et al. Human herpes simplex-1 and papillomavirus type 16 homologous DNA sequences in normal, potentially malignant and malignant oral mucosa. Eur J Cancer B Oral Oncology. 1993;29B:215-219.
25. Cornelia FA, Saper CD, Christensen RE, Park NH. Effect of DMBA on oral cancer development in hamsters with latent HSV-1 infections in trigeminal ganglia. Oral Surg Oral Med Oral Pathology. 1989;67:167-171.
26. Pilon L, Royal A, Langelier Y. Increased mutation frequency after herpes simplex virus type 2 infection in non-permissive XC cells. Journal of General Virology. 1985;66: 259-265.
27. Meurman JH. Infectious and dietary risk factors of oral cancer. Oral Oncology Journal. 2010;46:411-413.
28. Starr JR, Daling JR, Fitzgibbons ED, et al. Serologic evidence of herpes simplex virus 1 infection and oropharyngeal cancer risk. Cancer Research Journal. 2001;61:8459-8464.
29. Parker TM, Smith EM, Ritchie JM, et al. Head and neck cancer associated with herpes simplex virus 1 and 2 and other risk factors. Oral Oncology Journal. 2006;42: 288-296.
30. Eglin RP, Scully C, Lehner T, Ward-Booth P, et al. Detection of RNA complementary to herpes simplex virus in human oral squamous cell carcinoma. Journal Lancet. 1983;322(8353):766–768.
31. Gupta K, Metgud R. Evidences suggesting involvement of viruses in oral squamous cell carcinoma. Pathology Research International; 2013.
32. 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.
33. 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;2:10-14.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/24989