

Molecular Detection of Herpes Simplex Virus (1,2) in Oral Squamous Cell Carcinoma at Khartoum

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Abstract

Background: In oral squamous cell carcinoma (O.S.C.C) many factors are attributed in the etiology of it including environmental and genetic factors. An important role in the etiology is played by oncogenic viruses. The most commonly implicated viruses in oral squamous cell carcinoma were the human papilloma viruses, herpes group, adenoviruses, and hepatitis C virus. **Methodology:** An observational descriptive retrospective cross-sectional study was conducted at Khartoum Teaching Dental Hospital and the Department of Virology, Central Laboratory, Ministry of Science and Communication; in the period of 2014 and 2015 using Polymerase Chain Reaction (PCR). A total of 117 paraffin embedded tissue samples from oral squamous cell carcinoma were collected. DNA was extracted and HSV-1 & HSV-2 were detected using PCR. **Results:** 117 paraffin embedded tissues were examined to detect HSV-1 & HSV-2 DNA with PCR after the extraction of DNA. HSV-1 was detected in twenty two samples while HSV-2 was detected in eight cases. Fifty seven patients (48.7%) had S.C.C in the mandible and 22 patients (18.8%) in the maxilla. Seventeen patients (14.5%) had lesions in the tongue, and sixteen (13.7%) had it in the lower lip. There was significant increase of occurrence of HSV-1 and oral squamous cell carcinoma in the mandible ($p < .05$). **Conclusion:** Herpes viruses could be one of the factors that lead to oral squamous cell carcinoma as type 1 has a significant increase specially in the mandible.

Keywords

Oral Squamous Cell Carcinoma, HSV1, HSV2, PCR, Khartoum, Sudan

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1. Introduction

Oral squamous cell carcinoma is a major health problem in many parts of the world, with an incidence rate higher in developing countries, and with males being more affected than females [1].

The etiology of O.S.C.C is considered to be multifactorial and the factors influencing it include environmental factors, life style, infection agents and genetic alterations. The main predisposing factors are tobacco and alcohol abuse [2]. An important role in the etiology of many of them is played by

the oncogenic viruses [3]. The most commonly implicated viruses in O.S.C.C have been the human papilloma virus (HPV) [4, 5], herpes group [6], adenoviruses [7] and hepatitis c viruses [8, 9].

Herpes simplex belongs to a family of eight related viruses, including (HSV-1) and (HSV-2), varicella-zoster virus, Epstein-Barr virus, and cytomegalovirus [10]. HSV-1 is an important pathogen that causes a variety of clinical manifestations in humans, predomantly infects the oral

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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 [11, 12, 13, 14, 15, 16].

2. Material and Method

2.1. Study Site

This is a descriptive Cross Sectional study design chosen to survey HSV-1 and HSV-2 in oral squamous cell carcinoma patients presenting to Khartoum Teaching Dental Hospital, total coverage to excisional biopsies in the period from 2014-2015.

2.2. Sample Collection

117 Paraffin embedded blocks tumor specimens from all the excisional biopsies (2014-2015) from patients with squamous cell carcinoma were collected from (Histopathology Lab, Faculty of Dentistry, Khartoum University) during December 2015.

2.3. DNA Extraction

20- μ sections were cut from each tissue specimen block by the same person. To avoid cross-contamination, the microtome block was cleaned and the blade was replaced between specimens. Specimens were deparaffinized and DNAs was extracted using (Aidlab, china) according to manufacturer’s instructions.

2.4. DNA Amplification and PCR

PCR was performed by processing the extracted DNA with primers that are specific for HSV-1 and HSV-2 genes. The primers will be used for HSV1 are Forward: GTTAGGGAGTTGTTCCGGTCATAAGCT Reverse: TCGGCCATCTTGAGCATCC, the HSV2 primers are: Forward: GTCGGTGTGGTGTTCGGTCATAAGCT Reverse: GGCTGAATCTGGTAAACACGCTTC.

Thirty four thermal cycles were performed in ESCO 6.5 thermal cyclor after adjusting the programme: Initial denaturation 94°C for seven minutes, Denaturation 94°C for one minute, Annealing temp 57°C for two minutes, Extention 75°C for one minute and half. And final extention 75°C for seven minutes. After amplification, the PCR products were electrophoresed on 2% agarose gel containing ethidium bromide (0.5g/ml) and visualized under ultraviolet illumination.

2.5. Ethical Review

An ethical approval was obtained from the Research Ethics Committee of the faculty of dentistry & graduate college, the University of Khartoum.

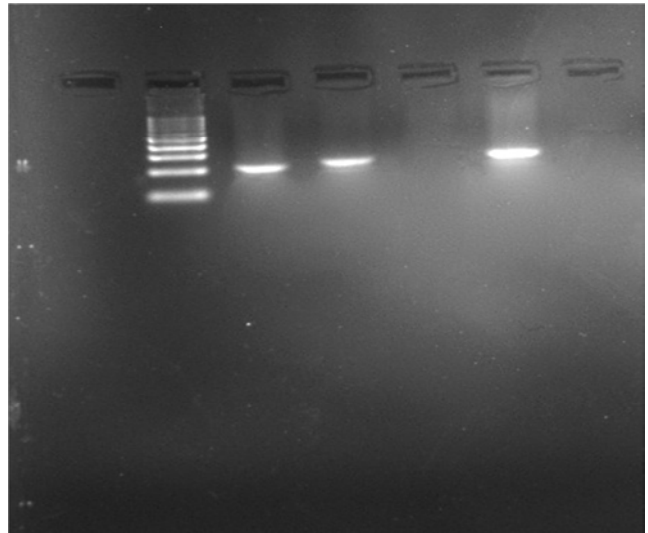


Figure 1. Examples of positive samples for HSV1 (Band length was 208 bp).

3. Results

A total of 117 paraffin embedded tissue from excised oral squamous cell carcinoma tumors in patients presented to KTDH hospital were enrolled in this study. 78 were males and 39 were females. Twenty two (18.8%) were smokers and 95 (81.2%) were not smokers. Snuff dippers were 70 (59.8%) and forty seven (40.2%) were not snuff dippers. Alcoholic consumers were only 9 patients (7.7%) as described in the graph:

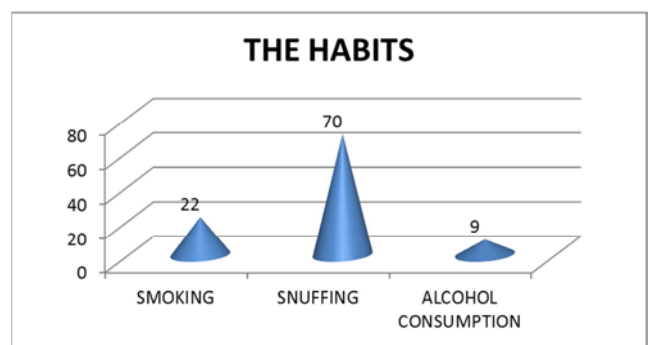


Figure 2. 70 patients were snuff dipper, twenty two smokers and nine patients were alcoholic.

Fifty seven patients (48.7%) were having the S.C.C in their mandible while 22 patients (18.8%) in the maxillae. Tongue S.C.C was in 17 patients (14.5). 16 patients showed the S.C.C in the lower lip equivalent to 13.7%. Histopathology grade was categorized to three categories: well differentiated O.S.C.C, moderately differentiated O.S.C.C and poorly differentiated O.S.C.C. Their percentages: 62.4%, 29.1%, 8.5% respectively as illustrated in figure.

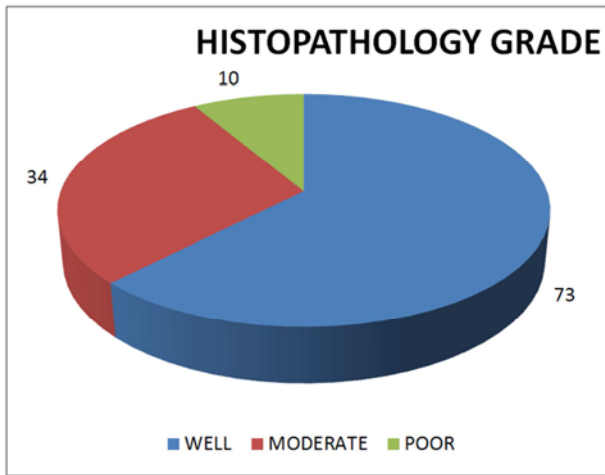


Figure 3. Most of the S.C.C cases were well differentiated histopathologically followed by moderately differentiated and the least number was having the poor differentiated histopathology.

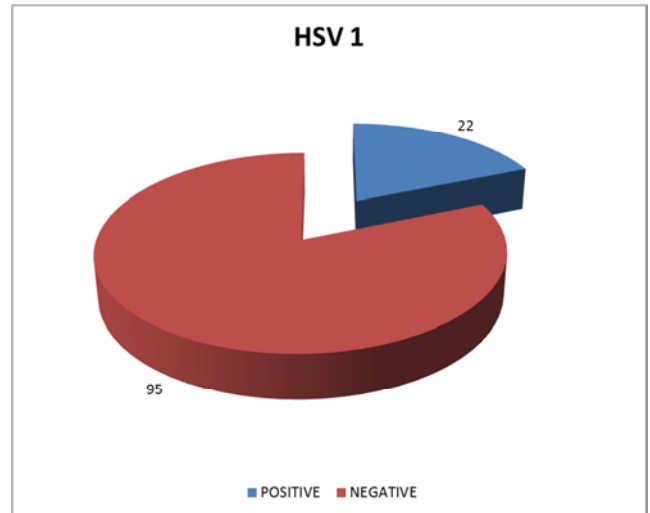


Figure 4. HSV-1 was positive in 22 cases.

HSV-1 was positive in 22 cases, HSV-2 was positive in 8 cases.

HSV 1 * MANDIBLE

Table 1. HSV-1 Mandible

Crosstab			MANDIBLE		Total
			YES	NO	
HSV 1	POSITIVE	Count	16	6	22
		% within HSV 1	72.7%	27.3%	100.0%
		% within MANDIBLE	28.1%	10.0%	18.8%
	NEGATIVE	Count	41	54	95
		% within HSV 1	43.2%	56.8%	100.0%
Total	Count		57	60	117
	% within HSV 1		48.7%	51.3%	100.0%
	% within MANDIBLE		100.0%	100.0%	100.0%

Table 2. Chi-square tests

Chi-Square Tests	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	6.252 ^a	1	.012		
Continuity Correction ^b	5.124	1	.024		
Likelihood Ratio	6.424	1	.011		
Fisher's Exact Test				.017	.011
Linear-by-Linear Association	6.198	1	.013		
N of Valid Cases ^b	117				

4. Discussion

Herpes simplex virus is a double-stranded DNA enveloped virus, when it infects cells the series of very complex event take place, the virus lives in two closely related forms, (HSV-1 and HSV-2). HSV 1 causes mainly oral and ocular infections, whereas HSV 2 causes mainly genital infections. Cells that are infected by HSV are destroyed. Gene maps of both HSV types have been developed and show that the

various functions of the two viruses are encoded by similar regions of the genome of each virus. [17] Induction of cellular proteins (such as heat shock proteins) has been studied as a possible mechanism for transformation by HSV [17, 18, 19]. Host cell shutoff is recognized as being another important factor that induced by HSV. The infected cell stops to synthesize cellular proteins, and cell RNA is degraded quickly. Another activity of HSV that might be related to cell transformation is stimulation of the replication of other viruses [20].

Jalouli *et al.* investigated the prevalence of HPV, HSV, and EBV DNA by PCR sequencing in brush biopsies obtained from patients with oral dysplasias and OSCC. Their findings illustrate that prevalence of HSV, HPV and EBV infections are common and may influence oral health and cancer development. It is not obvious that cancer risk is increased in infected toombak users. [21]. These results agree with the findings of the current research.

Eglin *et al.* examined biopsy specimens from patients with O.S.C.C by in situ hybridization for evidence of RNA complementary to HSV1 and HSV2. RNA complementary to HSV was found in over 50% of oral squamous cell carcinomas. While in this study it was 30%. [22].

Delavarian *et al.* investigated the presence of viruses in OSCC in Twenty one formalin-fixed, paraffin-embedded sections of young patients for the first time in Iranian population. Authors concluded that viruses had no important role in OSCC in young patients [23]. There is a difference between their result and the result of this study. It is possible that these differences can be attributed to the small size of sample and the targeted age which is 40 years and less, while in this study all ages were involved.

Mozhagan Mokhtari and Mozhdeh Beiraghadar in their study about the prevalence of HSV1 infection in O.S.C.C specimens in Alzahra and Kashani hospitals with polymerase chain reaction method in 2012-2013. 60 paraffin-embedded biopsies were analysed for the presence of HSV DNA using PCR. Only three samples were positive and they suggest that it doesn't play an important role in O.S.C.C [24]. The difference between the result of this study and the one mentioned by Mozhagan and Mozhdeh might be explained by different sampling techniques, divergent PCR methods and also the quality of the sample.

In summary, the aim of this study was to investigate the presence of HSV-1 DNA and HSV-2 DNA in OSCC patients attending Khartoum Teaching Dental Hospital. The prevalence of HSV-1 positive sample in this study was 18.6%, and HSV-2 is 6.8%. It shows that HSV-1 has an important role in OSCC because there was significant difference in mandible specifically.

The majority of OSCC patients in this study had a long history of smoking, alcohol consumption or both, therefore, it is concluded that these life styles may play an important role in this group of patients.

Because of the number of the positive specimens, more studies, especially case-control, with more specimens are needed in order to clarify the different aspects of HSV-1 involvement and its relationship to OSCC.

5. Conclusion

Oral cancer is an important cause of morbidity and mortality, especially in developing countries, and its prevalence may rise in the foreseeable future. The studies of virus-associated head and neck cancers have provided many critical 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. In this work, A total of 117 paraffin embedded tissue samples of oral squamous cell carcinoma were selected. 78 males, 39 females. Mean age of patients was 58 years. Twenty two (18.8%) were smokers and 95 (81.2%) were not smokers. Snuff dippers were 70 (59.8%) and forty seven (40.2%) were not snuff dippers. Alcoholic consumers were only 9 patients (7.7%). HSV-1 & HSV-2 were examined by conventional PCR, and the result was that type 1 was detected in 22% out of 117 cases, and type 2 in 8%.

These results warrant further studies to determine the possible role of viral infections and co-infections with other viruses as risk markers for the development of O.S.C.C. These viruses may provide targets for therapy and for diagnostic tests, and may widen The understanding about the mechanisms by which the tumors develop.

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