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Research Article

Is There Link between Kaposi's Sarcoma-Associated Herpes Virus (KSHV) in Group of Male Oral Squamous Cell Carcinoma Patients with Established Risk Factors in Non-Endemic Sri Lanka? Evidence from a Case-Control Study

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ABSTRACT

Head and Neck Cancers (HNSCCs) are established as looming public health challenge and health economic burden as the 8th most common cancer type according to a 2020 global estimation. Of them, Oral Squamous Cell Carcinoma (OSCC) ranks as the number 1 histologically diagnosed type of oral malignancy with 90%-95% prevalence across the globe. The geographic and population specificity is common in oral cancer patients as well as patients infected with the Kaposi's Sarcoma-Associated Herpes Virus (KSHV). Hence, KSHV infections among immunosuppressive patients are higher in endemic areas than in non-endemic areas. Moreover, KSHV associated carcinomas are common among patients suffering from Acquired Immune Deficiency Syndrome (AIDS). Here we hypothesized that immunosuppression in oral cancer patients could elevate the transcription and active transmission of oncogenic γ-herpes viruses in oral cancer patients. The present study aimed to link the Kaposi's Sarcoma-Associated Herpes Virus (KSHV) in a group of male oral squamous cell carcinoma patients with established risk factors in non-endemic Sri Lanka. Incisional biopsies of cases and excisional biopsies of controls were collected, transported, stored, and dispatched as frozen tissues at -800°C. Then, DNA extraction from frozen specimens was done using Gentra Puregene Tissue kit (Qiagen, Germany), solid tissue protocol strictly adhering to the manufacturer's instructions. Then, real-time PCR technology was used to diagnose KSHV infection in these OSCC cases and FEP controls. The specific KSHV DNA fragment was not detected in 22 OSCC cases and 29 FEP control samples. Thus, a link between KSHV and oral cancer patients with established risk factors in non-endemic Sri Lanka was not found.

Keywords: Head and neck cancers; Kaposi's sarcoma associated herpes virus, Real-time PCR, Immunosuppression; Biopsy

INTRODUCTION

Head and Neck Cancers (HNSCCs) are re-emerging as a distressing public health menace as they rank as the 8th common cancer type according to the 2020 global estimation [1]. Oral squamous cell carcinoma is the commonest subgroup of head and neck squamous cell carcinomas consisted of the malignant neoplasms beginning from the lining mucosae of the lips and the mouth (oral cavity) comprising the anterior two-thirds of the tongue as defined by the WHO international classification of disease [2]. Amidst, effective prevention and control measures for oral cancer in Sri Lanka, this island contributes immensely to the global oral cancer burden like Papua New Guinea, Bangladesh, and Hungary, with the highest incidence rates in the Asia-Pacific region [3]. This neoplastic disease becomes a health economic burden with an unbearable cost of treatment modalities and palliative care activities, especially when the Sri Lankans economy collapsed. Even at this moment lip, tongue, and mouth cancer is the 1st among males in Sri Lanka, mainly due to lifestyle related modifiable risk factors. In the Indian

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Received: 26-Apr-2023, Manuscript No. IGOA-23-23758; **Editor assigned:** 28-Apr-2023, PreQC No. IGOA-23-23758 (PQ); **Reviewed:** 12-May-2023, QC No. IGOA-23-23758; **Revised:** 22-Jun-2023, Manuscript No. IGOA-23-23758 (R); **Published:** 28-Dec-2023, DOI: 10.35248/IGOA.23.8.219

Citation: Perera ML, Perera IR, Kandewatte RL (2023) Is There Link between Kaposi's Sarcoma Associated Herpes Virus (KSHV) in Group of Male Oral Squamous Cell Carcinoma Patients with Established Risk Factors in Non-Endemic Sri Lanka? Evidence from a Case-Control Study. Immunogenet Open Access. 8:219.

subcontinent and Sri Lanka, betel quid chewing with smokeless tobacco slake lime, and areca nut, smoking, and heavy alcohol consumption usually introduce well-known carcinogens established as the aetiological agents of oral cancers [4,5]. Human Papillomavirus (HPV) was also established as an aetiological agent for a subset of HNSCCs, especially in the oro-pharynx (C10) and tonsils (C09), especially among younger Caucasian males comparatively with high socio-economic status, non-smokers and none or light drinkers. Approximately, 20% of oral cancer cannot be explained by established aetiological agents may be due to infectious oncogenic agents. Hence, finding the possible link between oncogenic viruses and oral cancer still remains a grey area in most instances due to the fact that disappearance of circulating viral DNA after the integration into the host keratinocyte genome.

The Kaposi Sarcoma (KS) associated herpes virus KSHV also known as Human Herpes Virus 8 (HHV 8) discovered in 1994 [6]. This virus and Epstein Bar Virus (EBV) belong to the subfamily of Alpha viruses in the family of Herpesviridae. Moreover, both EBV and KSHV equipped with oncogenic and pathogenic properties. Hence, this endemic HHV-8 is established as the causative agent of uncommon neoplasms such as Multicentric Castleman's disease (MCD) and Primary Effusion Lymphoma (PEL) typically associated with HIV infected patients and the commonest HIV associated malignancy in the world. Molecular pathological evidence unveiled the mechanisms of oncogenesis of HHV-8, *via* molecular mimicry, viral encoded proteins are found to be activated by several cellular signaling cascades whilst evading immune surveillance [7]. Immunosuppressive conditions in oral cancer patients may provide an ideal opportunity to activate oncogenic viruses from their latent stage [8-11]. Viral infections due to Epstein Bar Virus (EBV), the other γ-herpes virus among OSCC cases reported previously in local regional and global contexts. Nevertheless, there are no published studies either on the status of HHV-8, in Sri Lankan OSCC patients with oral risk habits or the malignant transformation potential of benign oral mucosal lesions by KSHV. Against this backdrop, the present case control study aimed to detect a link between Kaposi's Sarcoma-Associated Herpes Virus (KSHV) in group of male oral squamous cell carcinoma patients with established risk factors in non-endemic Sri Lanka?

MATERIALS AND METHODS

Study design, sample size calculation, setting, and subjects

Present molecular epidemiology study was based on a multicenter field study in Sri Lanka. A representative sub sample of 29 OSCC cases and 25 FEP controls was selected from the main unmatched case control study as described previously adhering to stringent exclusion and inclusion criteria as described previously [12]. The scientific sample size calculation for the unmatched case control study was based on Kelsey, et al. Selected Oral and Maxillo-Facial (OMF) units across Sri Lanka were visited located in six provinces and written informed consent was obtained from each participant as described previously [13].

Risk habit profile

Pre-tested interviewer administered questioner was used to collect the details of oral risk habits and other sociodemographic information of study subjects as described previously.

Tissue sampling and DNA extraction

Deep tissue pieces (~3 mm^3) from excisional FEP biopsies were taken. Subsequently, DNA extraction from frozen samples (=800°C) was performed according to (solid tissue protocol) as described previously.

Real time PCR for HHV

β-globin PCR with the primers PCO3 and PCO4 was performed to ensure the quality of DNA and to confirm the absence of PCR inhibiting agents. The real time PCR assay was set up to amplify 106 bp of HHV-8, from previously published primer sequences as described previously. Then, the real time PCR was performed on a quant studio 6 real time machine with an initial step of hold stage of polymerase activation step at 950°C for 5 minutes, followed by 45 cycles of amplification (5 seconds denaturation at 950°C for 5 minutes; 30 seconds annealing (TM)/extension at 550°C) and melt curve stage of 3 steps (950°C for 10 minutes, 500°C for 10 minutes and 950°C for 15 minutes). Overall run duration was 72 minutes and 24 seconds. Amplicon detection was determined *via* RT-PCR screen and melt curve analysis.

RESULTS

The results presented here included histopathologically confirmed 29 of OSCC cases and clinically diagnosed 25 FEP Controls.

The distribution of cases and controls

Accordingly, highest number of cases 8 (27.6%) were collected from district hospital Kegalle and highest number of controls obtained from teaching hospital Kurunegala 4 (16.0%) (Figure 1).

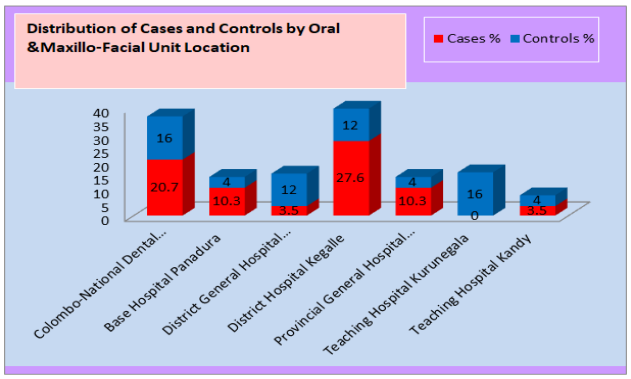

Figure 1: Distribution of cases and controls by oral and maxillo-facial unit location.

Sociodemographic profile

As indicates in Table 1, the mean ± SD age of cases was 61.62 ± 9.21 years where as the mean ± SD age of the controls was 49.96 ± 13.38 years and this difference in age groups was statistically significant ($p<0.05$).

Table 1: Distribution of cases and controls by sociodemographic profile.

Variables	Cases n=29	Controls n=25	p value	
Age mean ± SD in years	61.62 ± 9.21	49.96 ± 13.38	0.0001*	(p<0.05)
Gender	**N %**	**N %**		
Male	29 (100.0)	25 (100)		
Level of education	**N %**	**N %**		
No schooling	4 (13.9)	1 (4.0)	0.171**	(p>0.05)
Primary education	9 (31.0)	3 (12.0)		
Secondary education	7 (24.1)	10 (40.0)		
Above secondary education	9 (31.0)	11 (44.0)		
Total	29 (100.0)	25 (100.0)		
Occupation	**N %**	**N %**		
Farmer	15 (51.7)	8 (32.0)	0.014***	(p<0.05)
Skilled/Unskilled manual categories	12 (41.4)	7 (28.0)		
Clerical/Professional	2 (6.9)	10 (40.0)		
Total	29 (100.0)	25 (100.0)		

Note: *t-test to compare means of independent samples.

**Fisher's exact test to compare groups (cell counts <5).

***Chi Square of statistical significance.

Accordingly, there is a statistically significant association between the differences in tooth cleaning habit, missing teeth, mobile teeth and periodontal disease status (Table 2).

Table 2: Distribution of cases and controls by tooth cleaning habits and clinical indicators.

Variable	Cases n=29 N %	Controls n=25 N %	p-value
Tooth cleaning habit			

Tooth brush and tooth paste	14 (48.0)	24 (96.0)	0.0001*	(p<0.05)
Finger and charcoal/tooth powder	1(3.5)	0 (0.0)		
Brush with toothpaste/ charcoal	10 (34.5)	0 (0.0)		
Finger with charcoal	2 (7.0)	1 (4.0)		
Chewing stick	2 (7.0)	0 (0.0)		
Total	29 (100.0)	25 (100.0)		
Missing teeth mean ± SD	10.07 ± 9.95	3.96 ± 4.88	0.007"	(p<0.05)
Mobile teeth mean ± SD	3.45 ± 3.16	0.44 ± 1.12	0.001"	(p<0.05)
Decayed teeth mean ± SD	0.79 ± 1.72	1.20 ± 1.85	0.406"	(p>0.05)
Filled teeth mean ± SD	0.03 ± 0.19	0.16 ± 0.47	0.193"	(p>0.05)
Oral hygiene status#				
Good	3 (10.3)	13 (52.0)	0.004*	(p<0.05)
Fair	20 (69.0)	9 (36.0)		
Poor	6 (20.7)	3 (12.0)		
Total	29 (100.0)	25 (100.0)		
Periodontal disease status##				
Mild	4 (13.8)	17 (68.0)	0.0001**	(p<0.05)
Moderate	15 (51.7)	6 (24.0)		
Severe	10 (34.5)	2 (8.0)		
Total	29 (100.0)	25 (100.0)		
Site affected				
Buccal mucosa	19 (65.5)	21 (84.0)	0.122**	(p>0.05)
Tongue	10 (34.5)	4 (16.0)		
Total	29 (100.0)	25 (100.0)		
Histopathology				
Well-differentiated SCC	15 (51.7)	0 (0.0)	NA	
Moderately-differentiated SCC	14 (48.3)	0 (0.0)		
Fibro-Epithelial Polyps (FEP)	0 (0.0)	25 (100.0)		
Total	29 (100.0)	25 (100.0)		

Note: #Classified according to Simplified Oral Hygiene Index (OHI-S) of Green and Vermillion.
##Case definitions for periodontitis developed by Centre for Disease Control (CDC) periodontal disease surveillance workgroup.
" t-test for independent samples.

*Fisher's exact test of statistical significance.
**Chi-Square test of statistical significance.
NA: Not Applicable

Accordingly, there is a statistically significant association (p<0.05) between the differences among cases and controls on betel quid chewing, alcohol consumption, daily vegetable consumption and daily fruit consumption (Table 3).

Table 3: Distribution of the cases and controls by risk habit profile and daily vegetable and fruit consumption.

Variable	Cases n=29	Controls (n=25)	p-value	
	N %	N %		
Betel chewing habit				
Never	0 (0.0)	4 (16.0)	0.003*	(p<0.05)
Past	5 (20.0)	2 (8.0)		
Sometimes	1 (4.0)	7 (28.0)		
Daily	23 (76.0)	12 (48.0)		
Total	29 (100.0)	25 (100.0)		
Smoking habit				
Never	5 (17.2)	8(32.0)	0.418*	(p>0.05)
Past	8 (27.6)	6 (24.0)		
Sometimes	4 (13.8)	5 (20.0)		
Daily	12 (41.4)	6 (24.0)		
Total	29 (100.0)	25 (100.0)		
Alcohol consumption				
Never	3 (10.3)	3 (12.0)	0.001*	(p<0.05)
Past	6 (20.7)	2 (8.0)		
Sometimes	4 (13.8)	16 (64.0)		
Weekly	16 (55.2)	4 (16.0)		
Total	29 (100.0)	25 (100.0)		
Daily vegetable consumption				
<5 portions	23 (79.3)	9 (36.0)	0.001**	(p<0.05)
≥ 5 portions	6 (20.7)	16 (64.0)		
Total	29 (100.0)	25 (100.0)		
Daily fruit consumption				

<5 portions	23 (79.3)	8 (32.0)	0.001**	(p<0.05)
≥ portions	6 (20.7)	16 (68.0)		
Total	29 (100.0)	25 (100.0)		

Note: **Chi-Square test of statistical significance.

*Fisher's exact test.

HHV-8 status in cases and controls

Neither OSCC cases nor FEP controls contained KSHV/HHV-8 DNA examined by HHV-8 q PCR assay (Figure 2).

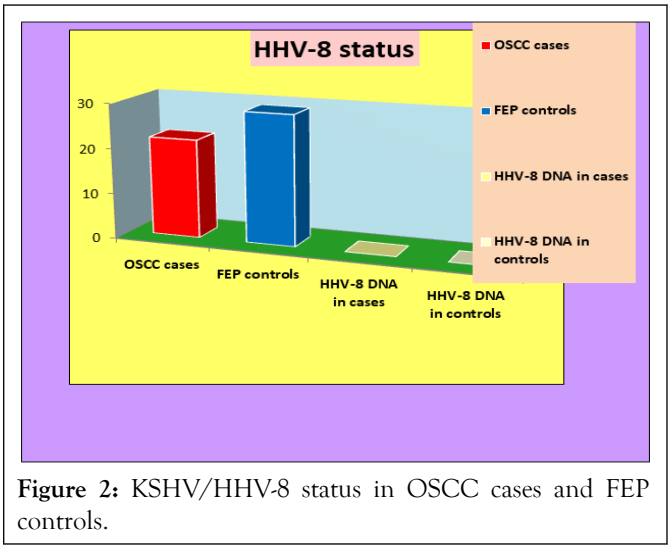

Figure 2: KSHV/HHV-8 status in OSCC cases and FEP controls.

DISCUSSION

To the author's knowledge, this is the 1st study conducted to find out possible link between Kaposi's Sarcoma-Associated Herpes Virus (KSHV) in group of male oral squamous cell carcinoma patients with established risk factors in non-endemic Sri Lanka. The epidemiological value of the present study is higher because a representative sub sample was obtained from the main sample to represent the vast majority of oral cancer patients in Sri Lanka, The majority of oral viral infections caused by Human Herpes Viruses (HHVs). Of, HHV-8 accounts for the aetiological agent of infectious diseases and malignancies in immunosuppressive patients, especially in HIV patients. Neither OSCC cases nor FEP controls contained KSHV/HHV-8 DNA examined by HHV-8 q PCR assay. A comparable finding was reported in a study conducted by Yang et al. and colleagues 13 as this virus was not detected in any of the 25 archival FFPE samples. HHV-8 was detected in one of 22 archival FFPE samples of head and neck squamous cell carcinomas in a separate study of HIV-positive patients 14. Speicher and colleagues 15 were able to describe an HIV-negative transplant recipient in whom MCD presenting as a Post-Transplant Lympho-proliferative Disorder (PTLD) is proven to be due to the recrudescence of HHV-8 [15].

Moreover, life-style related risk habits such as betel chewing heavy alcohol consumption, lack of micro nutrients by daily supply of sufficient quantity of fruits and vegetables, poor oral hygiene status due to inadequate tooth cleaning habits and periodontal disease status, of the OSCC cases could have significantly associated with oral carcinogenesis in these OSCC patients with low socio economic status of farmer and skilled/unskilled manual worker categories [16]. The hundreds of carcinogenic metabolites comprising aromatic nitrosoamines and poly cyclic aromatic hydrocarbons tetrahydropyridine present in smoked and smokeless tobacco, arecanut, slake lime and alcohol are well known carcinogens and aetiological agents of oral carcinogenesis [17]. However, it has been revealed that the KSHV infection is not ubiquitous but this virus can cause asymptomatic infections in young adults in endemic areas [18]. Furthermore, risk factors and transmission routes exhibiting considerable variations across populations in different geographic region which justify the negative finding of present study [19]. KSHV transmits *via* saliva during childhood in endemic areas and *via* homosexual contact in non-endemic countries. In contrast, the prevalence of EBV was 34 (64.2%) for overall subjects and significantly higher 21 (77.8%) for OSCC cases when compared 13 (50.0%) for FEP controls previously on the same subjects. This difference was statistically significant [20]. Hence, if KSHV presented at least one of these subjects it would have detected using optimized and standardized quantitative real time PCR technology. Hence, small sample size is probably not a limitation of the present study. Furthermore, Sri Lanka is a non-endemic country for these alpha viruses in the family of Herpesviridae.

CONCLUSION

There is no link between Kaposi's Sarcoma-Associated Herpes Virus (KSHV) in group of male oral squamous cell carcinoma patients with established risk factors in non-endemic Sri Lanka. However, descriptive cross-sectional studies among HIV and organ transplant Sri Lankan patients are recommended to find out the possible prevalence of this ɣ virus which can cause infections such as carcinomas.

ETHICAL APPROVAL

Ethical approval for present study was received from the faculty research committee, faculty of dental sciences, university of Peradeniya, Sri Lanka (FRC/ FDS/UOP/E/2014/32) and

Griffith university human research ethics committee, Australia (DOH/18/14/ HREC).

AUTHOR CONTRIBUTIONS

M. Perera, contributed to conception, design, data acquisition and interpretation, scientifically improved the manuscript; I. Perera, contributed to design, data acquisition, interpretation and statistical analysis.

ACKNOWLEDGMENTS

First and foremost special word of thanks goes to Prof. DJ Speicher for his unconditional contribution to optimizing and standardizing the detection of human herpesvirus by quantitative polymerase chain reaction and his valuable publications on KSHV associated infections and malignancies in immunosuppressive patients. Finally, I would like to pay my gratitude to Dr. DS, Ipe, Prof. GC Ulett, and Prof. NW Johnson for their valuable guidance and advice to make this molecular epidemiology study based on Sri Lankan subjects successful.

CONFLICT OF INTEREST

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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