Human Herpesvirus-6 and Epstein–Barr Virus Infections at Different Histopathological Grades of Oral Squamous Cell Carcinomas

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ABSTRACT

Background: The aim of this study was to determine the prevalence and viral load of Epstein–Barr virus (EBV) and Human herpesvirus-6 (HHV-6) in different histopathologic grades of oral squamous cell carcinoma (OSCC).

Methods: Forty-five formalin-fixed paraffin-embedded tissue section of OSCC patients were analyzed by quantitative real-time polymerase chain reaction for detection of EBV and HHV-6.

Results: The mean age of the patients was 58.6 years, 69% of whom were female, and 31% were male. Overall, the positive rate for EBV and HHV-6 were 16.7% and 27.1%, respectively; and the mean viral load EBV was 27.9×10^3 and 38.5×10^3 for HHV-6. No correlation was demonstrated between the viral load of EBV DNA ($P = 0.35$) and HHV-6 ($P = 0.38$) at the different OSCC histopathologic grades.

Conclusions: These findings neither lend support to the hypothesis that EBV and HHV-6 are directly involved in OSCC nor rule out the possibility that these viruses play an indirect role in carcinogenesis in this area.

Keywords: Epstein–Barr virus, herpesvirus-6, infection, oral cancer

Original Article

INTRODUCTION

Squamous cell carcinoma (SCC) is the most common malignancy of the oral cavity and includes 88% of mouth and lips malignancies.[1] Different factors have been attributed to cause oral SCC, among them internal factors such as systemic disease, malnutrition, general resistance, and iron deficiency anemia and external factors include tobacco and alcohol use, syphilis, poor oral hygiene, and sunlight (at vermilion) could be mentioned.[2]

Epstein–Barr virus (EBV) whose cell lines were determined by Tony Epstein and Yvonne Barr from Burkitt's lymphoma in 1965[3] belongs to the genus lymphocryptovirus of γ-herpesvirus family.[4] Its early exposure in infancy is usually asymptomatic, whereas it causes infectious mononucleosis in adolescence. In general, EBV infects more than 90% of the world adult population.[4] In B lymphocytes, EBV causes lytic infection, while,

in epithelial cells, it results in latent infection.[5] EBV gene expression alters the biological properties of the infected cells in latent and lytic infections and may result in cancer in humans.[6] This virus is involved in several human malignancies including Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma.[7]

Human herpesvirus-6 (HHV-6) was firstly extracted from peripheral blood mononuclear cells of the patients with AIDS and lymphoproliferative disorders by Salahuddin in 1986.[8] According to genetic characteristics, the virus is classified as a β-herpesvirus, although based on biological properties it can be placed in the γ-herpesvirus subfamily.[9] Primary infection occurs during the first 2 years of life and is usually associated with undifferentiated febrile illness, although some children show the classical manifestation of roseola infantum (exanthema subitum).[10] HHV-6 encodes the U24 protein which has been implicated in the pathology of multiple sclerosis.[11] This virus is associated with various oral lesions and is reported to be present in different cells; its reactivation in epithelial tumors of the oral cavity has also been shown.[12] Since recognition of the role of viral factors seems important in the development of oral SCC (OSCC) and the role of viruses in oral cancer may vary among different countries, the present study evaluated the frequency of EBV and HHV-6 viruses in OSCC samples in Zahedan, Sistan and Baluchestan Province located in the south-east of Iran.

METHODS

General materials

All the diagnosed OSCC samples of Department of Pathology, and Zahedan School of Dentistry from 2004 until 2013 were evaluated. The histopathologic grading samples were performed by two oral and maxillofacial pathologists through microscopy in a three-grade scale of I to III. High mature tumors resembling their tissue of origin, that is squamous epithelium, were graded as well differentiated SCC or grade I. In contrast, tumors with high nuclear and cellular polymorphism and very low or no production of keratin were classified as poorly differentiated SCC or grade III and tumors with a microscopic appearance between these two grades were classified as moderately differentiated SCC.[13] Demographic information was also obtained from the patients files.

Samples collection

This cross-sectional research protocol was approved by ethical committee of Research Deputy of the Zahedan University of Medical Sciences. Three sections were prepared from the formalin-fixed paraffin-embedded (FFPE) tissue blocks and were put in 1.5 ml microtubes for DNA extraction which was performed using the specific kit of RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE (Ambion, Carlsbad, California, USA). In brief, paraffin-embedded tissues are deparaffinized using xylene and wash the pellet twice with ethanol. Next, add protease digestion with an incubation time tailored for recovery of DNA. Nucleic acids are purified using a rapid glass-fiber filter methodology that includes an on-filter nuclease treatment and are eluted into water.

The DNAs were then evaluated using specific primers for HHV-6 and EBV of the AmpliSens® EBV/cytomegalovirus (CMV)/HHV-6-screen-FRT polymerase chain reaction (PCR) kit (Moscow, Russia). The PCR condition was showed in Table 1. The fluorescent quantitative real-time PCR amplification curve of HHV-6 showed in Figure 1.

Statistical analysis

Data analysis was run by SPSS software version 17 (Chicago, IL, USA). Data are presented as mean ± SD Chi-square, ANOVA, Kruskal–Wallis, Mann–Whitney tests and Spearman correlation coefficient were used for the comparison of statistical differences between the groups, and $P < 0.05$ was considered statistically significant.

Table 1: The PCR conditions amplification of EBV and HHV-6

Step	Temperature, °C	Fluorescent detection	Cycles
Hold	95	15 min	1
Cycling 1	95	5 s	5
	60	20 s	
	72	15 s	
Cycling 2	95	5 s	40
	60	20 s	
	72	15 s	

PCR=Polymerase chain reaction, EBV=Epstein-Barr virus, HHV-6=Human herpesvirus-6

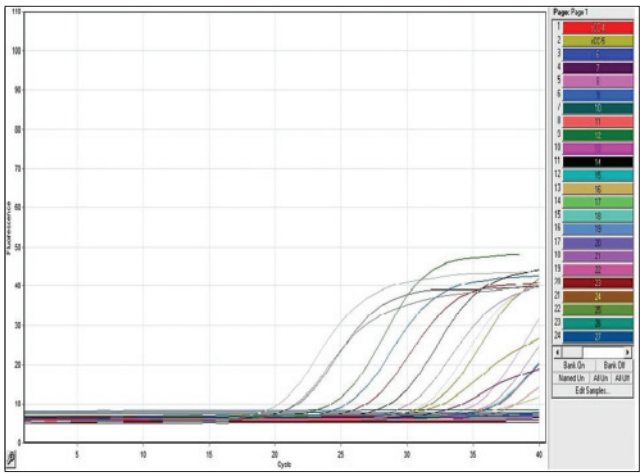

Figure 1: The fluorescent quantitative real-time polymerase chain reaction amplification curve of Human herpesvirus-6

RESULTS

The mean age of the patients whose samples were evaluated was 58.6 ± 13.9 years. Of 45 studied samples, 31 patients (69%) were female with a mean age of 56.4 ± 12.8 years and 14 patients (31%) were male with a mean age of 63 ± 15.3 years. The gender of three patients with OSCC was unknown.

Of all samples, 21 (43.8%) cases were from grade I, 19 (39.6%) grade II, and 8 (16.7%) grade III and no significant correlation was found between the mean age of the patients and the histopathological grades (ANOVA test, $P = 0.85$).

Epstein–Barr virus positive was found in 8 (16.7%) samples and HHV-6 positive in 13 (27.1%) samples. The mean viral loads of EBV and HHV-6 in different histopathologic grades of positive samples are shown in Table 2. No correlation was demonstrated between the viral load of EBV ($P = 0.35$) and HHV-6 ($P = 0.38$) at the different OSCC histopathologic grades.

According to Spearman correlation coefficient, a significant correlation was found between the viral load of EBV and HHV-6 in all cases ($r = 0.37$, $P = 0.009$), while no significant correlation was found between the viral load of EBV and HHV-6 in positive cases ($r = 0.001$, $P = 0.999$).

There was also no relationship between the viral load of EBV and HHV-6 in positive cases and patient's age, (Spearman correlation coefficient, $r = 0.156$, $P = 0.71$ and $r = 0.096$, $P = 0.78$ respectively). The viral loads of EBV and HHV-6 in positive samples are shown separately for two genders in Table 3. According to the Chi-square test, no significant statistical association was observed between the prevalence of HHV-6 and gender ($P = 0.48$). Due to the low number of EBV positive cases, it could not be analyzed.

Generally, 7.7% of OSCC samples were observed in lips, 7.7% in tongue, and 32.7% in the buccal mucosa, 1.9% in the floor of the mouth, 17.3% in the maxillary gingiva, and 32.7% in the mandibular gingiva. All OSCC samples in tongue were negative for EBV and HHV-6. Considering the lack of data in different locations, the viral loads only described in different location and data analysis was not performed [Table 4].

Table 2: Viral loads comparison of EBV and HHV-6 in different histopathological grades in positive samples

Grade	Viruses	
	EBV	HHV-6
Grade I		
Mean	32.8×10^3	40.7×10^3
n	4	7
SD	43.8	57.3
Median	13×10^3	16×10^3
Interquartile range	70.7×10^3	71×10^3
Grade II		
Mean	34×10^3	25.2×10^3
n	2	4
SD	18.4	12.8
Median	34×10^3	26.5×10^3
Interquartile range		23.7×10^3
Grade III		
Mean	12×10^3	57×10^3
n	2	2
SD	9.9	21.2
Median	12×10^3	57×10^3
Interquartile range		
Total		
Mean	27.9×10^3	38.5×10^3
n	8	13
SD	31.3	42.9
Median	18.5×10^3	18×10^3
Interquartile range	33.2×10^3	45.5×10^3
P value*	0.35	0.38

*Kruskal-Wallis test. SD=Standard deviation, EBV=Epstein-Barr virus, HHV-6=Human herpesvirus-6

Table 3: Viral loads comparison of EBV and HHV-6 in two genders in positive samples

Viruses	Gender	*n*	Mean	SD	Median	Interquartile range	*P* value
EBV	Male	4	33×10^3	43.9	14.5×10^3	73×10^3	1.0*
	Female	4	22.8×10^3	17.1	18.5×10^3	30.3×10^3	
HHV-6	Male	3	69.7×10^3	77.1	35×10^3		0.517*
	Female	9	31.9×10^3	26.7	18×10^3	45.5×10^3	

*Mann-Whitney test. SD=Standard deviation, EBV=Epstein-Barr virus, HHV-6=Human herpesvirus-6

Table 4: The viral load of EBV and HHV-6 distribution by location in positive samples

Location	Viruses	
	EBV	HHV-6
Labial mucosa		
Mean		11×10^3
n	0	1
SD		
Median		11×10^3
Interquartile range		
Buccal mucosa		
Mean	41.3×10^3	36×10^3
n	3	3
SD	49.7	1.4
Median	21×10^3	31×10^3
Interquartile range		
Floor of the mouth		
Mean		12×10^3
n	0	1
SD		
Median		12×10^3
Interquartile range		
Maxillary gingiva		
Mean	7.5×10^3	62×10^3
n	2	4
SD	0.71	70.8
Median	7.5×10^3	44×10^3
Interquartile range		131×10^3
Mandibular gingiva		
Mean	28×10^3	38.2×10^3
n	3	4
SD	16.5	27
Median	19×10^3	30×10^3
Interquartile range		48.75×10^3
Total		
Mean	27.9×10^3	38.5×10^3
n	8	13
SD	31.3	42.9
Median	18.5×10^3	18×10^3
Interquartile range	33.2×10^3	45.5×10^3

SD=Standard deviation, EBV=Epstein-Barr virus, HHV-6=Human herpesvirus-6

DISCUSSION

Oral cancer is the sixth most common cancer in the body including SCC as the most common one. SCC has different etiological causes and viruses which have been proposed as an etiological factors.[2].The present study aimed to evaluate the prevalence and viral load of EBV and HHV-6 infection in different histopathologic grades of OSCC.

In this study, the prevalence of EBV in OSCC was 16.7% which was higher than a study performed in the northeast of Iran.[14] It may be due to the difference of studied population, since they had only examined OSCC in patients <40 years of age.[14] The prevalence of EBV infection on OSCC has been reported from 0% to 100% in different areas of the world.[15-20] The reason for this difference may arise from geographic diversity of different samples. For instance, Tsuhako has reported different frequencies of EBV-positive OSCC in different regions of Japan.[21] To determine EBV in these studies, various techniques such as; PCR, *in situ* hybridization, southern blot analysis, and immunohistochemistry have been used. The difference in the prevalence has been attributed by some researchers to different sensitivities of the assessment techniques[15,20] and the statistical methods used.[16,22]

Epstein–Barr virus has also been proposed to be associated with malignancies such as Burkitt's lymphoma, nasopharyngeal carcinoma, gastric cancer, and salivary glands cancers.[23] EBV encodes several proteins with transformation potential including EBV nuclear antigens 2 and 3 (EBNA2 and EBNA3) and latent membrane protein 1 and 2 (LMP1 and LMP2).[24] *In vitro* studies have shown that LMP1 can inhibit p53-mediated apoptosis in epithelial cells.[23] The high prevalence of EBV DNA in tumoral tissues can be due to infiltration of

EBV-infected B lymphocytes and macrophages or the higher sensitivity of tumor keratinocytes to EBV infection altered by the immunological environment in the presence of the tumor.[25] Decreasing local, and general immunity can also increase the prevalence; accordingly, studies have reported high prevalence of EBV in clinically normal oral mucosa of immunocompromised patients and hence support this theory.[15]

Failure to detect EBV DNA in some studies has been described by the "*hit and run*" theory, according to which viral DNA is required only for malignancy induction, and it disappeared during uncontrolled cell cycles of the host[22] In some studies, the viral DNA has been isolated from patients with OSCC via PCR; however, the expression of viral proteins could not be determined.[21,25] Various descriptions have been proposed in this regard, for example, the identified viral genomes in carcinoma cells may remain un-translated, or they may originate from oropharynx and be present in the saliva and hence contaminate tumor samples without being a carcinogen.[22] It has been also proposed that the viral DNA may reside in infiltrating lymphocytes.[26] Thus, the role of EBV as a risk factor still remains controversial.

The mean viral load of EBV and HHV-6 in positive samples of OSCC was $27 \pm 31 \times 10^3$ and $38 \pm 42 \times 10^3$, respectively. According to the correlation of histopathologic grades of OSCC with EBV prevalence, various studies have obtained different results. For instance, no significant association has been reported between histopathologic grades of OSCC and EBV by Sand *et al*.[15] Despite stating the lack of correlation between histopathologic grades and EBV prevalence by Maeda and Shamaa *et al*., they observed higher EBV prevalence in lower histopathologic grades of OSCC.[23,27] Given the increased nuclear atypia in OSCC, Gonzalez-Moles *et al*. have shown a higher prevalence of EBV.[17] In contrast to these studies, Kobayashi *et al*. have reported a higher prevalence of EBV in higher histopathologic grades of OSCC, which was statistically significant.[16] In the present study, no significant correlation was observed between the mean viral load of EBV and histopathologic grades of OSCC in positive samples ($P = 0.77$).

Given the infectious nature of EBV and its tendency to spread, isolation of the virus from multiple lesions has raised the hypothesis of "field cancerization" in tumors of the head and neck.[28] This hypothesis was first proposed in 1953 and implies that the surrounding epithelium of tumor samples taken from the upper gastro-respiratory path has histopathologically changed.[29] However, further studies are needed to confirm this hypothesis.

The mean age of patients in the present study was 58 ± 13 years and consistent with the other studies;[15,23,27] no relationship was observed between age and the prevalence of EBV and the viral load. Although not statistically significant, the prevalence of EBV was higher in men than women. Maeda *et al*. did not find a significant correlation between the prevalence of EBV in OSCC and gender,[23] whereas Shamaa *et al*. reported a significantly higher prevalence in men.[27] Other cancers related to EBV such as Hodgkin's lymphoma, Burkitt's lymphoma, and nasopharyngeal carcinoma are also 2-3 times more common in men. This difference in EBV-related gastric cancers has been attributed to different exposures over a lifetime, occupational risk factors, and biological factors or hormones in both males and females. Based on information, testosterone suppresses the immune system, and thus men are more prone to infection and its outcomes.[30] Environmental factors such as the use of smokeless tobacco products can increase the risk of oral cancer through increased EBV replication and associated inflammatory processes in the oral cavity.[31] Perhaps higher prevalence of smoking among men in this region is due to the increased prevalence of this virus in OSCC of men. However, Sand and D'Costa *et al*. did not observe a significant association between chewing tobacco, snuff use, alcohol consumption, and smoking and the prevalence of EBV in OSCC samples.[15,18] In the present study, no significant correlation was found between OSCC location and EBV prevalence; this finding is consistent with the studies of Maeda and Sand *et al*.[15,23] However, Gonzalez-Moles *et al*. has reported a significantly higher prevalence of EBV in the lateral surface of the tongue.[17]

HHV-6 was identified in some malignancies such as oral cancer, cervical carcinoma, and

Hodgkin's and non-Hodgkin's lymphomas.[32] HHV-6 produces the oncoprotein of open reading frame-1 which binds to wild-type p53 and disrupts cell cycle regulation. Neoplastic transformation of nontumoral human epidermal keratinocytes by HHV-6 DNA has been also shown.[32] In the present study, the prevalence of HHV-6 in the examined samples was 27%. Using PCR, Yadav *et al*. reported the frequency of HHV-6 in OSCC in 1994 and 1997 as 67% and 57%, respectively,[33,34] which are much higher than our result. Significant increase of HHV-6 antibody levels in OSCC patients compared to controls indicates a possible role of HHV-6 in the pathogenesis of OSCC.[35] In the present study, no significant correlation was found between the mean viral load of HHV-6 and histopathologic grades of OSCC ($P = 0.72$). Similarly, in a study by Yadav *et al*., no correlation was observed between the presence of HHV-6 antigen and differentiation of OSCC through immunohistochemistry. Yadav *et al*. had studied the presence of HHV-6 in OSCC in three methods; the highest HHV-6 was found with immunohistochemistry and *in situ* hybridization and the lowest with PCR; the reason of which was attributed to the fragility and susceptibility of that part of DNA amplified by PCR due to formalin-fixation treatment.[34] There was no significant correlation between the viral load of HHV-6 in OSCC and age and gender in this study. In addition, no study examining the association of age, gender with the prevalence of HHV-6 in OSCC was found to be compared with ours.

HHV-6 is capable of activating other HHVs such as EBV, CMV, and papillomaviruses. Razavi *et al*. have reported a significant association between the presence of HHV-6 and EBV genomes in salivary gland neoplasms.[36] In addition, a significant correlation was suggested by Zhou *et al*. between HHV-6 and EBV infections and histopathologic progress of angioblastic T-cell lymphoma.[37] In the present study as well, a correlation existed between the mean viral load of EBV and HHV-6 in OSCC.

CONCLUSIONS

In total, a significant correlation was not found between the mean viral load of EBV and HHV-6 and histopathologic grades of OSCC. These findings, therefore, do not lend support to the hypothesis that EBV and HHV-6 are directly involved in OSCC. The data do not rule out the possibility that these viruses play an indirect role in disease pathogenesis that they use a 'hit and run' mechanism or are involved in a small minority of cases.

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REFERENCES

1. Sargeran K, Murtomaa H, Safavi SM, Vehkalahti M, Teronen O. Malignant oral tumors in iran: Ten-year analysis on patient and tumor characteristics of 1042 patients in Tehran. J Craniofac Surg 2006;17:1230-3.
2. Orbak R, Bayraktar C, Kavrut F, Gündogdu C. Poor oral hygiene and dental trauma as the precipitating factors of squamous cell carcinoma. Oral Oncol Extra 2005;41:109-13.
3. Sarid R, Gao SJ. Viruses and human cancer: From detection to causality. Cancer Lett 2011;305:218-27.
4. Saha A, Kaul R, Murakami M, Robertson ES. Tumor viruses and cancer biology: Modulating signaling pathways for therapeutic intervention. Cancer Biol Ther 2010;10:961-78.
5. Paydas S, Ergin M, Erdogan S, Seydaoglu G. Prognostic significance of EBV-LMP1 and VEGF-A expressions in non-Hodgkin's lymphomas. Leuk Res 2008;32:1424-30.
6. Tsao SW, Tsang CM, Pang PS, Zhang G, Chen H, Lo KW. The biology of EBV infection in human epithelial cells. Semin Cancer Biol 2012;22:137-43.
7. Li J, Mookerjee B, Wagner J, Flomenberg N. *In vitro* methods for generating highly purified EBV associated tumor antigen-specific T cells by using solid phase T cell selection system for immunotherapy. J Immunol Methods 2007;328:169-81.
8. Chen X, Zhang C. Inhibitory role of baicalin on human herpes virus type 6 *in vitro*. Procedia Eng 2012;37:75-8.
9. Luppi M, Barozzi P, Garber R, Maiorana A, Bonacorsi G, Artusi T, *et al*. Expression of human herpesvirus-6 antigens in benign and malignant lymphoproliferative diseases. Am J Pathol 1998;153:815-23.

10. Caserta MT, Mock DJ, Dewhurst S. Human herpesvirus 6. Clin Infect Dis 2001;33:829-33.
11. Tait AR, Straus SK. Phosphorylation of U24 from Human Herpes Virus type 6 (HHV-6) and its potential role in mimicking myelin basic protein (MBP) in multiple sclerosis. FEBS Lett 2008;582:2685-8.
12. Victória JM, Guimarães AL, da Silva LM, Kalapothakis E, Gomez RS. Polymerase chain reaction for identification of herpes simplex virus (HSV-1), cytomegalovirus (CMV) and human herpes virus-type 6 (HHV-6) in oral swabs. Microbiol Res 2005;160:61-5.
13. Neville BW, Damm DD, Allen CM, Bouqout J. Oral and Maxillofacial Pathology. St Louis: Saunders; 2009.
14. Delavarian Z, Pakfetrat A, Falaki F, Pazouki M, Pazouki N. The role of viruses in oral squamous cell carcinoma in young patients in Khorasan. J Appl Sci 2010;10:981-5.
15. Sand LP, Jalouli J, Larsson PA, Hirsch JM. Prevalence of *Epstein-Barr* virus in oral squamous cell carcinoma, oral lichen planus, and normal oral mucosa. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:586-92.
16. Kobayashi I, Shima K, Saito I, Kiyoshima T, Matsuo K, Ozeki S, *et al.* Prevalence of *Epstein-Barr* virus in oral squamous cell carcinoma. J Pathol 1999;189:34-9.
17. Gonzalez-Moles MA, Gutierrez J, Rodriguez MJ, Ruiz-Avila I, Rodriguez-Archilla A. *Epstein-Barr* virus latent membrane protein-1 (LMP-1) expression in oral squamous cell carcinoma. Laryngoscope 2002;112:482-7.
18. D'Costa J, Saranath D, Sanghvi V, Mehta AR. *Epstein-Barr* virus in tobacco-induced oral cancers and oral lesions in patients from India. J Oral Pathol Med 1998;27:78-82.
19. Mizugaki Y, Sugawara Y, Shinozaki F, Takada K. Detection of *Epstein-Barr* virus in oral papilloma. Jpn J Cancer Res 1998;89:604-7.
20. Shimakage M, Horii K, Tempaku A, Kakudo K, Shirasaka T, Sasagawa T. Association of *Epstein-Barr* virus with oral cancers. Hum Pathol 2002;33:608-14.
21. Tsuhako K, Nakazato I, Miyagi J, Iwamasa T, Arasaki A, Hiratsuka H, *et al.* Comparative study of oral squamous cell carcinoma in Okinawa, Southern Japan and Sapporo in Hokkaido, Northern Japan; with special reference to human papillomavirus and *Epstein-Barr* virus infection. J Oral Pathol Med 2000;29:70-9.
22. Hermann RM, Füzesi L, Pradier O, Christiansen H, Schmidberger H. Presence of human papillomavirus-18 and *Epstein-Barr* virus in a squamous cell carcinoma of the tongue in a 20-year-old patient. Case report and review of the current literature. Cancer Radiother 2004;8:262-5.
23. Maeda T, Hiranuma H, Matsumura S, Furukawa S, Fuchihata H. *Epstein-Barr* virus infection and response to radiotherapy in squamous cell carcinoma of the oral cavity. Cancer Lett 1998;125:25-30.
24. Gondivkar SM, Parikh RV, Gadbail AR, Solanke V, Chole R, Mankar M, *et al.* Involvement of viral factors with head and neck cancers. Oral Oncol 2012;48:195-9.
25. Kis A, Fehér E, Gáll T, Tar I, Boda R, Tóth ED, *et al.* *Epstein-Barr* virus prevalence in oral squamous cell cancer and in potentially malignant oral disorders in an eastern Hungarian population. Eur J Oral Sci 2009;117:536-40.
26. Cruz I, Van Den Brule AJ, Brink AA, Snijders PJ, Walboomers JM, Van Der Waal I, *et al.* No direct role for Epstein-Barr virus in oral carcinogenesis: A study at the DNA, RNA and protein levels. Int J Cancer 2000;86:356-61.
27. Shamaa AA, Zyada MM, Wagner M, Awad SS, Osman MM, Abdel Azeem AA. The significance of *Epstein Barr* virus (EBV) and amp; DNA topoisomerase II alpha (DNA-Topo II alpha) immunoreactivity in normal oral mucosa, oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). Diagn Pathol 2008;3:45.
28. Yen CY, Lu MC, Tzeng CC, Huang JY, Chang HW, Chen RS, *et al.* Detection of EBV infection and gene expression in oral cancer from patients in Taiwan by microarray analysis. J Biomed Biotechnol 2009;2009:904589.
29. Ha PK, Califano JA. The molecular biology of mucosal field cancerization of the head and neck. Crit Rev Oral Biol Med 2003;14:363-9.
30. Murphy G, Pfeiffer R, Camargo MC, Rabkin CS. Meta-analysis shows that prevalence of *Epstein-Barr* virus-positive gastric cancer differs based on sex and anatomic location. Gastroenterology 2009;137:824-33.
31. Jenson HB, Heard P, Moyer MP. Evaluation of the effect of smokeless tobacco purified products and extracts on latent *Epstein-Barr* virus. Toxicology 1999;133:35-42.
32. Leite JL, Stolf HO, Reis NA, Ward LS. Human herpesvirus type 6 and type 1 infection increases susceptibility to nonmelanoma skin tumors. Cancer Lett 2005;224:213-9.
33. Yadav M, Chandrashekran A, Vasudevan DM, Ablashi DV. Frequent detection of human herpesvirus 6 in oral carcinoma. J Natl Cancer Inst 1994;86:1792-4.
34. Yadav M, Arivananthan M, Chandrashekran A, Tan BS, Hashim BY. Human herpesvirus-6 (HHV-6) DNA and virus-encoded antigen in oral lesions. J Oral Pathol Med 1997;26:393-401.

35. Liu JL, Xu ZK, Zhao Y, Jin Y, Dai YM, Yao K. Relationship between human herpesvirus 6 infection and oral squamous cell carcinoma. Zhonghua Shi Yan He Lin Chuang Bing Du Xue Za Zhi 2003;17:334-6.
36. Razavi M, Monirifard M, Sharif S, Khodaian N. Epidemiology of herpes human virus 6 and *Epstein-bar* virus infections in salivary gland neoplasms in Isfahan, Iran. Iran Red Crescent Med J 2010;12:572-5.
37. Zhou Y, Attygalle AD, Chuang SS, Diss T, Ye H, Liu H, *et al.* Angioimmunoblastic T-cell lymphoma: Histological progression associates with EBV and HHV6B viral load. Br J Haematol 2007;138:44-53.

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