

# 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

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## Abstract

**Background:** Although tobacco, alcohol abuse are well-recognized risk factors for oral squamous cell carcinoma (OSCC), there is evidence to indicate that herpes simplex virus (HSV) may also play some inducing role. The purpose of this study was to investigate the presence of HSV in Iranian patients with OSCC using the polymerase chain reaction (PCR).

**Materials and Methods:** Biopsies of OSCC were obtained from 60 patients, 54 males and 6 females, aged between 36 and 80 years old. Paraffin-embedded, histologically confirmed specimens were analyzed for the presence of HSV DNA using PCR.

**Results:** Only three samples (5%) was positive, suggesting that HSV may not play an important role in this group of patients.

**Conclusions:** The prevalence of HSV-1 positive sample in this study was 5%. It shows that HSV-1 has no important role in OSCC.

**Key Words:** Herpes simplex virus, oral squamous cell carcinoma, polymerase chain reaction

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## INTRODUCTION

Oral cancer is a major cause of mortality and morbidity and is one of the top ten cancers in the world, with wide differences in geographic distribution.<sup>[1,2]</sup> The majority of these cancers are squamous cell carcinoma. More than 274,000 new cases of oral squamous cell carcinoma (OSCC) are being diagnosed worldwide annually.<sup>[3]</sup> They often develop after the age of 50, with a peak incidence in the sixth decade of life and

stand for approximately 5% of cancers in male and 2% in female.

The risk factors include tobacco associated intra-oral carcinogens and alcohol, which may take part in a synergistic role in oral tumorigenesis. From relative risk factors of alcohol and tobacco, it has been suggested, that the majority of oral cancers are preventable. In one recent study tobacco accounted for 77% of oropharyngeal carcinoma cases in the

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examined population, alcohol for 52%, low vegetable consumption for 52%, and the combination of the three for nearly 85% of all cases.<sup>[4]</sup> The fact that viruses can play a role in cancer development was established approximately 100 years ago, when Peyton Rous demonstrated that sarcomas of chickens could be transmitted by the virus that came to be known as the Rous sarcoma virus.<sup>[5]</sup> In the 1930s it became apparent that tumors of rabbits could be transmitted by Shope papillomavirus.<sup>[6]</sup> The fields of RNA viral oncology and DNA viral oncology came from these observations on animals, and it is now realized that many human cancers can be attributed to viruses.

In a comprehensive review of the literature<sup>[7]</sup> the overall prevalence of human papilloma virus (HPV) in squamous cell carcinomas of the head and neck was found to be approximately 25%. The prevalence was higher in oropharyngeal carcinomas (35.6%) than in oral carcinomas (23.5%).

The herpes simplex viruses (HSV), have been investigated in the past years for likely associations with cancers in human being. Levels of antibody to HSV-1 in patients with oral cancer than in control subjects have been reported to be higher.<sup>[4]</sup> These antibodies were largely of the IgA and IgM classes, and patients with the highest levels of anti-HSV IgM had a shorter survival than other patients.<sup>[8]</sup> A combination of HSV seropositivity and a history of cigarette smoking was associated with a higher risk of oral cancer than would be expected from a purely additive effect.<sup>[9]</sup> Preliminary reports have been indicated the presence of both viral DNA and RNA of HSV in oral cancers.<sup>[10,11]</sup> Except for the fact that HSV can transform some animal cells to a malignant phenotype *in vitro*, the explanation for the relationship between OSCC and HSV could be a confounding variable.<sup>[12]</sup> Furthermore, HSV has shown synergistic carcinogenic activity in combination with chemicals *in vivo*.<sup>[13]</sup> Unfortunately, the association between OSCC and HSV is difficult to study, because transformed cells by HSV do not express specific virus antigens, and also do not retain any specific genes of the virus.<sup>[14]</sup> Instead it seems likely that the transformation is due to the mutagen action of virus, and a region of the viral genome has been defined, which raises the mutation frequency in cultured cells.<sup>[15]</sup> This may results in some features of malignancy, but not all.<sup>[16]</sup> Neither the mutations nor the phenotypic changes are adequately specific to use as a marker by which a herpes-induced malignancy could be diagnosed.<sup>[17]</sup>

Recent years have seen little progress in the study of malignant potential of HSV. The polymerase chain reaction (PCR) assay is a technique that has several

advantages above other methods. It can detect the viral presence in early infections and requires only a small quantity of biological material. PCR detection of HPV, Epstein–Barr virus (EBV), and HSV is highly sensitive and specific, and can supplement the clinical detection of virus-associated oral lesions.<sup>[18]</sup> Because of the herpes virus subtypes can be vary in different parts of the world, and there have been few studies in Iran about prevalence of HSV in OSCC, this study was conducted to determine the prevalence of HSV in OSCC (in isolation or combination with tobacco and alcohol) by PCR technique.

## MATERIALS AND METHODS

A total of 60 specimens of patients with OSCC were investigated for the presence of HSV-1 infection. The specimens used in this study were retrieved from two university hospital sources.

Clinical data and information related to lifestyle were obtained from clinical charts and personal communication.

The resected tissues were frozen immediately after collection and stored at  $-80^{\circ}\text{C}$  until used. Histopathological confirmation of specimens were performed by a pathologist according to WHO-TNM classification of oral cavity carcinoma.<sup>[19]</sup> A single pathologist reviewed randomly selected cases to confirm the diagnosis.

DNA was extracted from formalin-fixed paraffin block of tissues with the tissue extraction kit and added 20–30  $\mu\text{L}$  of elution buffer according to volume of samples. Extracts were keep at  $-20^{\circ}\text{C}$ . Mixture of PCR were added to all samples except target DNA (5  $\mu\text{L}$  PCR buffer, 4  $\mu\text{L}$   $\text{MgCl}_2$ , 2.5  $\mu\text{L}$  forward primer (5'CATCACCGACCCGGAGAGGGAC3'), 2.5  $\mu\text{L}$  reverse primer (5'GGGCCAGGCGCTTGTGTTGTA3'), 1  $\mu\text{L}$  polymerase enzyme, 30  $\mu\text{L}$  Sterile Water). Then 1  $\mu\text{L}$  target DNA were added. Thermal cyler were regulated according PCR thermal program and all samples were put in it. Negative control was prepared (PCR mixture without target DNA).

The PCR products were put in a 2% agarose electrophoretic gel containing ethidium bromide (0.5 g/mL) and visualized under ultraviolet illumination.

## RESULTS

A total of 60 cases of OSCC were utilized in the study. From 60 cases diagnosed in our centers (Alzahra and Kashani University Hospitals) between 2012

and 2013, 56 cases were presented in patients older than 40 years of age. The range of the age of these patients was 36–80 years old with mean age of 53.1 years. Fifty-four (90%) of our patients were male and the most common site of involvement was buccal mucosa (21 out of 60 or 35%).

All patients used tobacco, alcohol or both. Forty-eight (80%) and 23 (38.3%) cases were active smoker and alcoholic, respectively.

The DNA was extracted from all of our samples. Viruses were detected only in 3 cases (5%) by PCR analysis. The age of these patients were 54, 62 and 48 years. Two cases were smoker and another one was alcoholic and smoker.

## DISCUSSION

Herpes simplex virus is a double-stranded DNA enveloped virus. When it infects cells the series of very complex events 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. HSV causes a severe primary infection, followed by a latent infection that may be followed by recurrent 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.<sup>[20]</sup> The only viral function that is not in a related location between the two types is the ability to transform cells.

Induction of cellular proteins (such as heat shock proteins) has been studied as a possible mechanism for transformation by HSV.<sup>[20-22]</sup> Host cell shutoff is recognized as being another important that induces by HSV. The infected cell stops to synthesize cellular proteins, and cell RNA is degraded quickly.<sup>[23]</sup>

Another activity of HSV that might be related to cell transformation is stimulation of the replication of other viruses.<sup>[24]</sup>

El Sissy searched for possible presence of HSV-2 protein in OSCC, and in normal oral mucosa, using immunohistochemical peroxidase-antiperoxidase technique.<sup>[25]</sup>

This study showed HSV-2 is an initiator in early neoplastic changes in well-differentiated OSCC that becomes denatured and consequently not evident in less highly differentiated tumors.

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 is common (HSV less than others) and may influence oral health and cancer development.<sup>[26]</sup>

Delavarian *et al.* investigated the presence of viruses in OSCC in young patients for the first time in Iranian population.<sup>[27]</sup> Twenty-one specimens of patients under 40 years with clinical diagnosis of OSCC were evaluated for presence of HPV, EBV, HSV-1, and cytomegalovirus virus (CMV). From 21 specimens, viruses were detected only in three cases. All specimens were negative for HPV and CMV. Two samples were positive for EBV and the third one was co-infected with EBV and HSV-1. Authors concluded that viruses had no important role in OSCC in young patients. Further researches are needed to clarify this role and to identify other possible risk factors.

In summary, the aim of this study was to investigate the presence of HSV-1 DNA in OSCC patients attending university hospitals of Isfahan University of Medical Sciences. The prevalence of HSV-1 positive sample in this study was 5%. It shows that HSV-1 has no important role in OSCC.

This is similar to a study by Jalouli *et al.* in 2010.<sup>[28]</sup> In another study by Jalouli *et al.* which was done in eight different countries showed different prevalence of HSV and other viruses in OSCC. For HSV the prevalence range was between 15% and 55%. The highest prevalence was in UK.<sup>[29]</sup>

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

Because of the low 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.

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## Conflicts of interest

There are no conflicts of interest.

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