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Human papillomavirus in saliva of patients with oral squamous cell carcinoma

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Abstract

Objective: The aim of this study was to evaluate the presence of human papillomavirus (HPV) in saliva rinses of patients with oral squamous cell carcinoma (OSCC)and to analyze the possibility of using saliva as a diagnostic method for screening high-risk patients. Study design: The saliva sample of 22 patients with oral squamous cell carcinoma and 20 age-sex matched healthy controls were obtained. The presence of HPV 6, 11, 16, 18, 31, and 33 was evaluated by polymerase chain reaction (PCR). Results: In 40.9% of the patients and in 25% of the controls, the saliva was shown to be positive for HPV. In 27.3% of the patients and in 20% of the controls, the saliva was shown to be positive for HPV16; and none of the controls, except one patient was shown to be positive for HPV 18. Neither patients nor controls were positive for HPV 31 and 33. These differences were not statistically significant. Conclusions: The results of this study were unable to support the detection of HPV in saliva rinses as a diagnostic method for OSCC.

Key words: *Oral squamous cell carcinoma, human papilloma virus, PCR.*

Introduction

Oral squamous cell carcinoma (OSCC) accounts for over 90 percent of oral cancers (1). Established etiological factors of OSCC included cigarette smoking, heavy alcohol abuse, betel nut chewing, and smokeless tobacco use. However, a growing group of patients, including a large proportion of young adults and women, have no known tobacco or alcohol exposure. Increasingly researches have focused on identifying possible viral etiologic factors such as oncogenic human papilloma virus (HPV) (2).

The absence of definite early warning signs for most head and neck cancers suggests that sensitive and specific biomarkers are likely to be important in screening high-risk patients (3). A number of molecular markers have been used to detect these tumors with varying degrees of specificity and sensitivity. No reliable or clinically applicable marker has been shown to universally identify oropharyngeal squamous cell carcinoma or tumor aggressiveness. Therefore, development of a saliva-based screening test for oropharyngeal cancer may be able to detect early stage tumors before development of clinical symptoms (4).

Application of a real-time PCR technique in determination of HPV DNA level in head and neck tumor and premalignant tissues as well as in serum has been evaluated. Recent studies investigated HPV DNA in saliva rinses from patients with OSCC, but there is a controversy (5).

The aim of this study was to detect HPV in saliva of patients with OSCC by PCR in order to evaluate the possibility of using saliva rinse as a simple method of HPV detection in OSCC.

Material and Method

Patients and sample collection

Twenty two histopathologically confirmed OSCC patients participated in the study. The patients suspicious to OSCC were referred to Oral Medicine Department, Faculty of Dentistry, Tehran University of Medical Sciences and Meraj Institute. For each case, a healthy individual was selected from individuals visited oral medicine clinic for routine dental care as control that was matched for age, sex, and in some extent smoking condition. History of any head and/or neck lesion was considered as an exclusion criterion for the control participants.

The trial was performed in accordance with the Declaration of Helsinki and subsequent revisions (6) and approved by ethics committee at Tehran University of Medical Sciences. The details of the study were explained to all participants and written informed consents were obtained before entering into the study.

Corresponding pretreatment saliva and control saliva were collected by 10ml normal saline rinses that was gargled and expectorated. Samples were centrifuged (10 min, 3000 × g, 4C min) and supernant were discarded. Lysis buffer (10mM Trish-HCL, pH 8, 1mM MEDTA, 1% SDS, 1M NaCl) and proteinase K were added on the cell pellets and were incubated in 56˚C for 2 hours. The standard phenol-chloroform extraction and the ethanol precipitation were used for DNA purification and the pelleted DNA was resuspended in 50-100 µ1/41 of tridistilled sterile water (7). In order to determine the quality and quantity of the isolated DNA, each DNA was analysed by electrophoresis on 0.8% agarose gels stained with ethidium bromide and spectrophotometrically.

Polymerase chain reaction (PCR)

DNA samples were amplified with the GP5+ and GP6+ primers targeting the L1 open reading frame of broad spectrum HPV genomes (7). In all samples, the constitutively expressed SRY gene was amplified first to confirm the adequacy of the extracted DNA and PCR amplification. DNA from Caski and Hella cells was used as positive control in all preliminary PCR runs. Positive samples were further analyzed with type specific HPV primers to identify high risk (HPV 16 and 18), intermediate risk (HPV 31 and 33), and low risk (HPV 6 and 11). The results of genotype were verified by restriction fragment length polymorphism (RFLP) which used restriction enzymes BamHI, DdeI, HaeIII, HinfI, Pst I, RsaI and Sau 3AI (8). CaSki cell line (containing a high copy number of HPV 16) and HeLa cell line (containing HPV 18) were all received from Magdalena Grce.

Statistical analysis

Statistical analysis was performed using SPSS 11.5 software. Chi-Square and Fisher exact were used for data analysis. $P<0.05$ was considered to be statistically significant.

Results

Twenty two patients with oral squamous cell carcinoma and twenty healthy individuals were enrolled in this study. The case group included 13 females (59.09%) and 9 males (40.01%) with mean age of 64.2 ± 14.9 years and the control group included 12 females (60%) and 8 males (40%) with mean age of 64.4 ± 15. Five subjects from the case group (22.72%) and four subjects from the control group (20%) were smoker by the time of text and had history of smoking for at least 20 years. The involved sites in this study was tongue (n=10; 45.45%), alveolar ridge (n=4; 18.18%), floor of the mouth (n=3; 13.6%), buccal mucosa (n=2; 9.09%), lips (n=2, 9.09%), and gingivae (n=1; 4.5%)

The saliva of nine patients (40.9%) and five controls (25%) were positive for HPV. The table 1 compares the presence of HPV types in saliva of oral squamous cell carcinoma patients and healthy individuals. Although the presence of all types of HPV in saliva of oral squamous cell carcinoma patients was higher than control group but none of these differences were statistically significant.

Table 1. Comparison of HPV types in the saliva of oral squamous cell carcinoma patients and healthy individuals.

Type of HPVs	Patients (N=22)	Controls (N=20)	P value
HPV 6/11	3 (13.6%)	1 (5%)	0.341
HPV16	6 (27.3%)	4 (20%)	0.426
HPV 18	1 (4.5%)	0 (0%)	0.524
HPV 31/33	0 (0%)	0 (0%)	_

Discussion

A link between human papillomavirus and squamous cell carcinoma of the head and neck was suggested more than 20 years ago (9). Investigators showed a strong association between hig-risk-HPV infection and OSCC in Mexican, Sweden, Japanese, and Chinese populations (10-12). The role of high-risk oncogenic HPV in premalignant and malignant oral lesions has been an issue of extreme controversy with conflicting data reported by numerous studies. Its prevalence reported in OSCC vary from <5% to 100% (13). This wide range may due to a variety of reasons such as the choice of primers used in the PCR, inherent differences in the populations being studied, and the methods used for HPV detection (14). PCR techniques are very sensitive and in the absence of a quantitative method such as Real-Time PCR, insignificant virus (with no role in carcinogenesis) can be identified (9). In terms of sensitivity and specificity, in situ detection techniques of HPV in paraffin-embedded tissue sections of OSCC (using in situ PCR and in situ hybridization PCR) are more powerful in detecting HPV than the liquid-phase PCR methods (14). In contrast, Enzyme Linked Immune Assay (ELISA) lacks acceptable sensitivity in detecting clinically meaningful HPV infections (9).

In this study, the amount of genomic DNA obtained from saliva was about 2µg and sufficient for evaluation of HPV, in accordance with previous researches (15). HPV 16 was the most detectable virus in patient's salivary samples. This result was similar to other investigations that reported most prevalence and most carcinogenesis role for HPV 16 (2, 5, 16) but no relationship was found between HPV DNA test results from saliva rinses and OSCC. Herrero et al. (17), also, did not find a correlation between HPV presence in oral brush and head and neck tumor tissues. In contrast, in a study by Zhao et al. (5), it was shown that HPV 16 DNA in saliva rinses can reflect HPV 16 status of primary head and neck squamuos cell carcinoma. They found site specific preponderance of HPV 16 presence in oropharynx tumors as reported by other investigators (2,11,18). They suggest that quantitative analysis of HPV 16 in salivary rinses have 99% specificity but its significant expense and high rate of false-positive results make this test difficult to apply to a broad population. Smith et al. (19) demonstrated the statically significant association between the detection of HPV-high risk (HPV-HR) types in oral exfoliated cells and the presence of HPV-HR types in tumor tissue. They suggested that HPV testing of oral rinses may be predictive of an HPV-related head and neck cancer.

HPV 33 and 31 that were high risk for cervical cancers (20), were not found in saliva samples of neither squamous cell carcinoma patients nor healthy individuals.

In this study, saliva was collected by 10ml gargled and expectorated normal saline rinses that was similar to the study conducted by Zhao et al. (5) and Smith et al. (19). Furrer et al. (16) used superficial scrapes and demonstrated that in potentially malignant and malignant oral lesions, HPV DNA is more likely to be detected in superficial scraps than in biopsy specimens.

The results of this study, probably because of small sample size did not show that detection of HPV in saliva rinses could be used as a diagnostic method for OSCC. However, further studies are needed to approve or disapprove this method as a diagnostic one for OSCC.

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