International Journal of Advanced Biotechnology and Research (IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-8, Issue-3, 2017, pp1793-1798
http://www.bipublication.com

**Research article**

# An Investigation for the Presence of Human Papilloma virus in Oral Squamous Cell Carcinoma: a Pilot Study from Babol County, North of Iran

**Shima Nafarzadeh[1], Mohammad Mahdi zakarya[2*], Farzin Sadeghi[3] and Yousef Yahyapour[4]**

[1]Assistant Professor, oral and maxillofacial pathology,
Dentistry school, Babol University of Medical Sciences, Babol, Iran.
[2]Dental Student, Faculty of Dentistry, Babol University of Medical Sciences,Babol, Iran.
[3]Cellular and Molecular Biology Research Center,Health Research Institute,
Babol University of Medical Sciences, Babol, Iran.
[4]Infectious Diseases & Tropical Medicine Research Center,
Babol University of Medical Sciences, Babol, Iran.
*Corresponding Author: Mohammad Mahdi Zakarya, E-mail: mehdi.zakarya@yahoo.com

## ABSTRACT

**Introduction:** Oral Squamous cell carcinoma (OSCC) is the most common oral cavity cancer, with various etiologic factors contributing to it. In recent years, human papillomavirus (HPV) has been recognized as one of the possible causes of OSCC . The aim of this study is to evaluate the prevalence of HPV in OSCC in Babol County, the most populated county in the Mazandaran province,North of Iran .

**Materials and methods:** This study was performed on 21 samples of OSCC and 30 Oral Irritation Fibroma (OIF) samples, all of which were paraffin blocks and analyzed by SYBR Green real time PCR for the precence of HPV.

**Findings:** In total,51 orall samples (21 with OSCC and 30 OIF) wereexmined, In OSCC group 12 (57.1%) were male and 9 (42.9%) were female. The mean age of the OSCC subjects was 60.95±12.25 years. The HPV were found 4 out of 21 (19.04%) OSCC samples, of which 50% were males and 50% were females. In the malignant cases, they also had 52.4% (11) grade 1, 33.3% (7) grade 2 and 14.3% grade 3. In OIF group only one sample was positive for HPV

**Conclusion:** Generally it seems that, HPV plays a role in the OSCC etiology. However, due to the controversy of studies, this requires further studies.

**Key words:** (HPV) Human papillomavirus (OSCC) Oral squamous cell carcinoma, PCR

## INTRODUCTION:

Oral squamous cell carcinoma (OSCC) is the 11th most common cancer in the world, and accounts for 94% of all cancers in the mouth. Women show less annual incidence than men in any age group and the overallmale to female ratio is 3 to 1. like many other carcinomas, the risk of intraoral cancers especialy in men increases with age increasing. SCC is a multifactorial disease. In any case, both external and internal factors may be effective. Internal factors such as malnutrition, anemia, iron deficiency and external factors such as tobacco, alcohol, syphilis, and sun rays about Vermillion of lip carcinoma (1 ,2). Candidate infection, especially hyperplastic, oncogenes viruses and immune status, puts patients on the list of oral cancer cases. Today, it seems that only HPV viral viruses appear to play a role in the development of OSCC. HPV is also plays

role not only in the development of oral carcinomas, but also in the development of pharyngeal, laryngeal, esophageal, cervical, Vulva and penis carcinomas. Subgroups 16, 18, 31 and 33 HPV are most commonly associated with dysplasia and SCC (1, 2). Meanwhile, there is evidence of the virus's role, which is rising but has not yet been proven. These viruses can potentially control the ability of the host in proliferation and eliminate cell proliferation, thereby facilitating malignancy changes. Today it seems that HPV play a role in mouth carcinoma. The virus also affects not only the development of oral carcinoma, but also the development of pharyngeal, laryngeal and cervical carcinomas. (3, 4 and 5) Despite of improvements in the treatment and pathogenesis of oral cancer, the survival rate has not significantly increased over the last few decades and has remained at a range of 50-59%.Therefore, early diagnosis and prevention are needed to improve the patient's overall health (1, 2).

Human papillomavirus is a double-stranded DNA virus from the papilloma viride family. HPV tends to proliferate in epithelial cells of the skin and mucous membranes. (6) HPV is a major source of death in the United States and around the world. (7) This virus can cause various types of warts and cancers, particularly cancers, including uterine cancer, anal vagina, and subtype of neck and head cancers in humans. (6) 16 HPV are associated with oropharyngeal cancers. (8 , 6)

polymerase Chain Reaction (PCR) is generally referred to as partial DNA or RNA enhancement to the extent that it is observed by simple and commonly used laboratory methods. The ability of PCR to increase the nucleic acid in the test sample results in the rapid and specific identification of the cell type or microorganism desired in the sample. In addition to the use of PCR in laboratory diagnosis of diseases and the identification of cell types, this feature puts it as a reliable and sensitive tool for scientific research. Real-Time PCR can provide instantaneous monitoring of PCR response, and this technique has a higher sensitivity and accuracy than conventional PCR. In this diagnostic system, a fluorescent substance is released during the reaction, proportional to the product level of each PCR cycle, and its fluorescence is detected by a detector and recorded on a computer in a reproduction curve. The real-time PCR detection range is higher than conventional PCR and one of its applications is to determine the exact level of viral factors. (9 and 10) So far, no research has been done on the prevalence of this virus in OSCC in the North of Iran. Therefore, the purpose of this study was to investigate the prevalence of HPV in OSCC in the north of the country so that if the virus is introduced as a pathogenesis of this disease, maybe can provide prevention of this disease.

## MATERIALS AND METHODS

### Patients and Tissue Specimens

The current cross-sectional study included 51 formalin-fixed paraffin-embedded resection specimens with 21 oral squamous cell carcinoma (OSCC) and 30 Irritation fibroma (IF) histopathologic diagnosis. Tissue specimens were collected from Department of Oral and Maxillofacial Pathology, School of Dentistry, affiliated to Babol University of Medical Sciences. The medical records of these subjects were reviewed in order to obtain demographic characteristics including age, gender, residence and marital status, information regarding anatomical localization of the specimens and tumor grade. All experiments were performed according to the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki. This study was approved by the Ethical Committee of Babol University of Medical Sciences, and for all subjects, written informed consent was obtained.

### DNA Extraction

Ten µm thick tissue sections were deparaffinized according to a previously described procedure . DNA was extracted from each tissue sample, using DNA Extraction Mini Kit from Tissue

(YektaTajhizAzma, Tehran, Iran) according to the manufacturer's instructions. The quality and quantity of purified DNA was determined using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA). In addition, DNA integrity in each tissue sample was evaluated using human RNase P gene (RPP30) amplification based on a previously described procedure. Sterile microcentrifuge tubes containing only reaction mixtures were processed simultaneously with the tissue samples as a DNA extraction negative control.

**HPV Detection by Real Time polymerase chain reaction**

HPV DNA detection was performed using the qualitative Real Time PCR with L1 General Primers (GP5+ and GP6+) using a Rotor-Gene Q real-time PCR system (QIAGEN GmbH, Hilden,Germany). Real-time PCR was performed in a 25 µL reaction mixture containing 500 ng of extracted DNA, 12.5 µL SYBR Green qPCR Master Mix 2X (YektaTajhizAzma, Tehran, Iran) and 5pmol/µL of each primer. For amplification of HPV L1 region, the real-time PCR cycling conditions were as follows: 10min at 95˚C, followed by 40 cycles of 15 sec at 95˚C, 30sec at 48˚C and 30sec at 72˚C . To confirm amplification specificity melting curve analysis were carried out at temperatures between 65˚C and 95˚C, with temperature increasing at a rate of0.5˚Cs-1 .

**STATISTICAL ANALYSIS**

Statistical analyses were done by SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). The $\chi 2$-test was utilized to assess associations between categorical variables. P value of $\leq 0.05$ was considered to be statistically significant.

**FINDINGS:**

This study was performed on 21 samples of OSCC and 30 OIF samples, In OSCC group 12 (57.1%) were male and 9 (42.9%) were female. The mean age of the subjects was 60.95 ± 12.25 years. The mean age of men was 57.7 ± 12.77 and the mean age of women was 65.56 ± 10.45. All people with OSCC were married, of which 81% (17 people) had urban life and 19% (4 people) had rural life. The HPV was found in 4 (19.04%) samples, which 50% were male and 50% were female. All HPV-infected people had urban living. The frequency of HPV in OSCC is based on the demographic variables in Table 1.

**Table 1:** The prevalence of HPV in OSCC in terms of demographic variables

| | | Positive HPV | Negative HPV |
|---|---|---|---|
| Age | 50-35 | 0 | 3 |
| | 65-51 | 3 | 10 |
| | 80-66 | 1 | 4 |
| Sex | Male | 2 | 10 |
| | Female | 2 | 7 |
| marital status | Married | 4 | 17 |
| | Single | 0 | 0 |
| Residential Location | Urban | 4 | 13 |
| | Rural | 0 | 4 |
| City Location | Amol | 1 | 5 |
| | Babol | 1 | 6 |
| | Babolsar | 0 | 3 |
| | Mahmudabad | 1 | 1 |
| | Ghaemshahr | 0 | 2 |
| | Savadkooh | 1 | 0 |

All biopsied specimens approved from OSCC and OIF by the pathologist before doing experiment. The places in the mouth that biopsy performed, are listed in Table 2. the most common site of OSCC was buccal

vestibule (38.1%). However, according to chi-square test, there was no significant difference in the incidence of HPV infection among different locations (p = 0.065). In the case of lesions malignancy, 52.4% (11) had grade 1, 33.3% (7) grade 2 and 14.3% grade 3. People with type 3 malignancies were more than 60 years old and in women more than men. It was also more frequent in the buccal vestibule. 75% of HPV positive cases had malignancy grade 2 and 25% had grade 1 malignancy.

**Table 2:** Frequency of OSCC in different oral regions in the study group and in the case of HPV

| HPV-positive cases | Frequency | The number of patients | Lesion location |
|---|---|---|---|
| 1 | 4.8% | 1 | The soft palate |
| 1 | 4.8% | 1 | edentulous Ridge of maxilla |
| 1 | 14.3% | 3 | Lower lip |
| 1 | 28.6% | 6 | Lateral border of toungue |
| - | 38.1% | 8 | Buccal vestibule |
| - | 4.8% | 1 | edentulous Ridge of mandible |
| - | 4.8% | 1 | Ventral surface of toungue |

Statistical analysis of the relationship between histopathologic grade and HPV-infected specimens was investigated, which according to P = 0.223, there was no significant relationship between them. Each of the HPV-infected specimens was biopsied at various parts of the mouth (soft palate, Edentulous ridge in maxilla,lateral border of tongue, lower lip).

The demographic data of OIF individuals is presented in Table 3. However, there is no significant difference between age groups, gender, marital status and place of residence in terms of positive HPV in OIF. thehpv was only found in one sample that was a 46 years old male living an urban life.

**Table 3:** Demographic characteristics of OIF individuals

| | | Positive HPV | Negative HPV |
|---|---|---|---|
| Age | 33-18 | 0 | 11 |
| | 49-34 | 1 | 11 |
| | 66-50 | 0 | 7 |
| Gender | Male | 1 | 13 |
| | Female | 0 | 16 |
| marital status | Married | 1 | 22 |
| | Single | 0 | 7 |
| Residential Location | Urban | 1 | 16 |
| | Rural | 0 | 13 |

Table 4 shows the relationship between OIF and HPV according to the location of the lesion. According to Table 4, there was no significant correlation between OIF and HPV according to the location of the lesion (p = 0.93, df = 7, x2 = 2.41). Based on the results, HPV positive in the OSCC (19%) was higher than the HPV positive in OIF (3%).**Table 4:** The relationship between OIF and HPV according to the location of the lesion.

| | | Site of lesion | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Buccal vestibule | Edentulous ridge in mandible | Edentulous ridge in maxilla | floor of the mouth | gingiva | hard palate | lateral border of the tongue | lower lip | |
| HPV state | negative | 6 | 2 | 2 | 2 | 8 | 3 | 3 | 3 | 29 |
| | positive | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 |
| Total | | 6 | 2 | 2 | 2 | 9 | 3 | 3 | 3 | 30 |

**DISCUSSION & CONCLUSION:**

in terms of global spread, oral cancers are one of the most common cancers, and in studies of oropharyngeal and oral cancers, form about 3% of all cancers. If oral cancers, nasopharyngeal, pharynx, larynx, sinus, and salivary glands are added to this figure, they account for about 5% of total body cancer. In men, oral cancer is 4%, and in women it is 2%, and oral cancer accounts for 2% of total cancer deaths in males and 1% in women.(11 , 12) Today, there are many reports of the presence of human skin papillomavirus in the development of head and neck cancers. The purpose of this study was to investigate the prevalence of human papillomavirus in OSCC in the northern population of the country, so if the HPV is introduced as a pathogenesis of this disease, it may be a possible basis for preventing the disease. Oral cancer is a disease associated with age, and about 95% of cases are seen in people over the age of 40 and the incidence of illness increases after 40 years for every decade, but the average age of the diagnosis is about 60 years (13). In the study The average age of the whole population was 60.95 ± 12.25. The most commonly locations that involved oral cancers are the tongue, oropharyngeal region, and the mouth floor. Lips, gums. The posterior side of the tongue and the palate is less affected. Primary bone marrow SCC is rare but the tumor may be caused by epithelial cells or epithelial lesions such as cysts and ameloblastoma (13). The present study showed that the most common site of OSCC was buccal vestibule (38.1%). Pourjajorm H. et al., In a study, evaluated some of the characteristics of OSCC patients referring to Dentistry Faculty and Omid Hospital in Mashhad from October 2002 to October 2003. In their study, the most common place in the tongue and the buccal mucosa was same with our study (14). In the present study, HPV virus was found in 4 (19.04%) samples, of which 50% were male and 50% were female. As seen in North America, about 60% of studies have found the incidence of HPV in less than 35% of OSCC cases.

In Europe, 50% of the prevalence studies are less than 40%, and 25% of cases have reported prevalence rates of between 40% and 60%. (15) Thomson et al., in a study of Chinese patients with tonsillectomy SCC did not show any positivity for the papillomavirus in any of the 16 Chinese patients, while in the Australian patients with the same method (PCR) Were 46% positive (16). Also, 52.4% had lesions with grade 1, 33.3% grade 2 and 14.3% grade 3. However, in the study of Pourjajorm, most of the lesions were Grade 3 and Grade 2(14). The results of present study contradicted with previous studies results. The results of this study showed that HPV positive cases in OSCC (19%) were more than HPV positive in IF (3%). Holladay et al., in South Carolina in 1993, 39 cases of SOCC and 6 natural mucus were examined by PCR methods. Of 39 oral SCCs, 7 cases (18%) were positive and from 6 control cases only 1 case (17%) was positive. (17) which is consistent with the results of this study. What seems to be large number of studies in different parts of the world such as North America, South America, Europe , Asia and the East, HPV is developing SCC in oral cavity that can be considered as an etiologic factor, but its prevalence in studies different from 0 to 95%. Although some said that the reasons for this distinction are because of differences in method of work, but in the study of the methodology, almost all studies were performed by Southern blot hybridization or PCR. Therefore, it seems that these differences are more than geographical differences. although some correlate this to the use of chewing tobacco in eastern asia , such as india and taiwan, the figures show a significant diffrence with the north of iran.in Conclusion this study shows that in the north of iran HPV exists in the samples of OSCC and it may be directly as a risk factor or association with other factors, However this requires further studies.

**REFERENCE:**

1.Neville BW, Damm DD, Allen CM, Bouqout JE. Oral & Maxillofacial Pathology.3thed. Philadelphia: Saunders co;2016. p.409-421, 782-788.

2.Regezi JA, Sciubba JJ, Jordan RCK. Oral pathology-clinical pathologic correlation.6thed. Philadelphia: W.B Saunders co; 2012. p.219-221, 321-324.

3.Neville BW, Damm DD, Allen CM, Bouquot JE. Oral and Maxillo facial Pathology, 3th ed., Saunders, Missouri, 2009: 276-277.

4.Elango KJ, Suresh A, Erode EM, Subhadradevi L, Ravindran HK, Iyer SK, et al. Role of human papilloma virus in oral tongue squamous cell carcinoma. Asian Pacific journal of cancer prevention : APJCP. 2011;12(4):889-96.

5.Khovidhunkit SO, Buajeeb W, Sanguansin S, Poomsawat S, Weerapradist W. Detection of human papillomavirus in oral squamous cell carcinoma, leukoplakia and lichen planus in Thai patients. Asian Pacific journal of cancer prevention : APJCP. 2008;9(4):771-5.

6.Karen C, Carroll, Jeffery A, et al .Jawetz, Melnick&Adelberg's Medical Microbiology, 27th ed , 2015:631-634

7.Ault KA. Epidemiology and natural history of human papillomavirus infections in the female genital tract.Infectious diseases in obstetrics and gynecology. 2006;2006 Suppl:40470.

8.Hantz S, Alain S, Denis F. [Human papillomavirus prophylactic vaccines: stakes and perspectives]. Gynecologie, obstetrique&fertilite. 2006;34(7-8):647-55.

9.Espy MJ, Uhl JR, Sloan M, Buckwalter SP, Jones MF, Vetter JDC, et al. Real-Time PCR in Clinical Microbiology: Ap-plications for Routine Laboratory Testing. ClinMicrobiol Rev 2006; 19(1(: 165-256.

10.Mackay IM, Arden KE, Nitsche A. Survey and Summary Real-Time PCR in Virology. NUCLEIC ACIDS RES 2002; 30(6(:1292-1305. 11) Siegel MA. Premalignant Lesions. In: Ord RA, Blanc Cheart RH, editors. Oral Cancer: Dentist's role in diagnosis, management, rehabilitation and prevention. 1st ed. Chicago: Quintessence Publishing CO; 2000. P. 49.

12.Andrew K, Lee AK, Alexander K. Role of alcohol and tobacco in the etiology of head and neck cancer: A case - control study in the Doubs region of France. Eur J Cancer 1995; 31:301- 302.

13.Epstein JB. Oral cancer. In: Greenberg MS, Glick M, editors. Burket's oral medicine.Diagnosis and treatment. 10th ed. New York: BC Decker; 2003. P. 194-234.

14.Hassan Hussein Jajarm, Seyed Amir Al-Dawood. Identify some characteristics of oral SCC patients referred to dental school and Omid hospital in Mashhad from October 1381 to Persian date Mehr 1382. Journal of Dental School Mashhad University of Medical Sciences, 1384, Vol. 29, No. 3

15.S. Sargolzaei, Kaykhaee MR et al. Relationship between different genotypes of human papillomavirus oral squamous cell carcinoma. Journal of Dental School ShahidBeheshti University of Medical Sciences, Volume 23, Number 3, 1384.

16 .Carol H, Thompson AB. Absence of HPV in Tonsillar SCC from Chinese patients.Am J Pathol 2003: 2185-2186.

17 .Miller CS, Zeuss MS, White DK, et al: Detection of HPV DNA in oral carcinoma using polymerase chain reaction together with in situ hybridization. Oral Surg Oral Med Oral Pathol 1994;77:480