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Original Article

Prevalence of Oral Human Papilloma Virus in Healthy Individuals in East Azerbaijan Province of Iran

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Abstract

Background: Human papilloma virus causes benign and malignant abnormalities in different part of the body. The link between high risk types of HPV and some anogenital and aerodigestive tract cancer is well established. Oral HPV infection plays a role in developing oropharyngeal squamous cell carcinoma. We studied the prevalence of oral HPV in healthy individuals and its relative risk factors.

Methods: Saliva samples of 114 healthy subjects were collected for HPV DNA analysis. Volunteers completed questionnaires and signed a written consent. For data analysis descriptive statistic, chi square test and odds ratio was used.

Results: The frequency of oral HPV in healthy individuals was 6.1 %(seven participant).The most frequent type was HPV-18 in five of them.HPV-6 and HPV-66 each was detected in one case. Relation of oral HPV positivity to demographic features and risk factors was not statistically significant.

Conclusions: The prevalence of oral HPV infection in our community is the same as many other communities of developing countries, stressing that HPV-18 were the dominant type.

Keywords: Human Papilloma Virus (HPV), Oral Cancer, Saliva

Introduction

Human papilloma virus (HPV) a member of papilloma virus causes infection in epithelial skin cells and mucous membrane (1-4). HPV plays a role in developing benign and malignant disease in different parts of the body like anogenital area, esophagus, conjunctiva, and head and neck. Low risk types of HPV such as type 6,11,40,42,43 cause benign lesion, and high risk types 16,18,31,33,35,45 are related to malignant proliferation of epithelial cells. However, only persistent infection with high risk HPV may lead to precancerous lesions and finally cancer (4- 9). The role of high – risk type HPV is well established in more than 90% of cervical cancer in women and nearly 25% of distinct subset of Head and Neck Squamous Cell Carcinoma (HNSCC) (6,10-12). The link between HPV infection and oropharyngeal Squamous Cell Carcinoma (SCC), is more obviously determined (13, 14). In several studies HPV Deoxyribonucleic

acid (DNA) were detected in approximately 50% of oropharyngeal SCC particularly tonsils and base of tongue (6, 11-13, 15-18). Like cervical cancer, HPV-16 and HPV-18 have been the most common type detected in a HPV-related head and neck squamous cell carcinoma during decades (11,19,20). Several investigators believe this affinity is maybe due to resemblance between cervical and oropharynx epithelium (6,18).

Transmission of HPV occurs easily by any kind of sexual contact (6, 10, 14). All of the sexual active men and women can be infected with HPV. Intercourse is not the sole transmission route; even kissing could be a possible way for HPV transmission (7,8,21). Some investigators consider oral sex may be the main pathway for oral HPV infection (6,8,13).

The prevalence of HPV infection is increasing in developed countries. Center for Disease Control (CDC) has reported that 20 million people in the United States are infected with HPV (10,21). American social health Association has estimated that about 75-80% of Americans will be infected with HPV in some points of their lives before the age of 50 (1). According to many studies, individuals who are having more sexual partners and high-risk sexual behaviour including oral sex have increased chance for being infected by HPV (5-7, 10, 14, 15, 22). Recent case-control studies postulated that high risk HPV in oral mucosa can increase the risk of the distinct type of oropharyngeal SCC (1,5,6,12,16). In spite of decreasing prevalence of head and neck cancer accumulating data have demonstrated prevalence of HPV-related HNSCC are rising (7,13,14),that may be due to increasing high-risk sexual behaviour in young adults for having safe sex (6,8,12-14). Considering the important role of oral HPV in this subgroup of HNSCC (1, 16), several studies investigated the prevalence of oral HPV in healthy individuals, but their results have been highly controversial (5, 23, 24), still, limited information exists about the frequency and natural history of oral HPV in healthy people. Because of the positivity of documented data about HPV prevalence and its high-risk types in healthy subjects, this study was designed, primarily to determine the prevalence of oral HPV infection and its high-risk types and second to investigate the demographic and behavioural risk factors related to oral HPV in our district.

Materials and Methods

Participants in the study were one hundred fourteen cancer – free individuals aged 16-61year who referred to Shahid Ghazi Tabatabaei Hospital of Tabriz University of Medical Sciences, for different purposes. All participants completed a self administered questionnaire with information about demographic characteristics, cigarette smoking, alcohol consumption, sexual practice, and the numbers of sexual partners and signed a written informed consent. Volunteers were referred to dentist for dental examination and evaluating oral health.

Because of social and religious limitation in our community, some questions were not answered and 59 persons did not go to dentist. Saliva samples of participants were collected and sent to the laboratory for DNA analysis.

Laboratory Methods

DNA was extracted using salting out method and to control for DNA quality, the GAPDH gene was amplified in all samples. PCR was performed in a final reaction volume of 25 mL, containing 10 mL of template DNA, 2.5 mL 10X PCR buffer, 2 mL MgCl2 (50 mM), 1 mL dNTPs [100 mM], 3 mL of My09 Primer (10ìM), 3 mL My11 Primer (10mM) and 0.5 mL of Taq DNA Polymerase (5 U/mL). The PCR conditions were as follows: preheating for 5 min at 94ºC was followed by 40 cycles of 1 min at 94ºC, 1 min at 55ºC and 1 min at 72ºC and a final extension of 7min at 72ºC (25,26).

HPV detection by PCR was carried out in a nested-PCR system, using the primers MY09/11 and GP5+/6+. Nested-PCR was performed in a final volume of 60 mL, containing 10 mL of the first reaction, 6 mL 10X PCR buffer,3 mL MgCl2 (50 mM), 1 mL dNTPs [100 mM], 6 mL Primer GP5+ (10mM), 6 mL Primer GP6+ (10mM)and 0.5 mL of Taq DNA Polymerase (5 U/mL).The

PCR conditions were as follows: preheating for 4 min at 94ºC, 2 min at 40 ºC and 2 min at 72 ºC was followed by 43 cycles of 1 min at 94ºC, 2 min at 40ºC and 1 min and 2 min at 72ºC and was followed 1 min at 94ºC, 2 min at 40 ºC and 4 min at 72 ºC. Amplification products obtained from HPV- positive cases subjected to direct sequencing. Finally, for genotyping of HPV a complementary analysis of sequences obtained from Blast was performed using the website of http://www.ncbi.nlm.nih.gov/blast.

Results

Total tobacco use was described in one pack-year, (smoking one pack in each day for at least one year). None of the participants had smoked other than tobacco. Cut point for smoking was never, <1-pack year and >1-pack year. Alcohol consumption defined as the average number of drinks per week for more than one year.We used descriptive statistics, chi-square test and odd-ratio with confidence interval 95% for the data analyzing. Association was considered to be statistically significant for a two sided $P<0.05$.

One hundred and fourteen saliva samples were used for HPV DNA analysis. Enrolled subjects were between aged 16-61 with the median age of 31.61 years (SD: ± 10.047). Table 1 shows demographic and risk factor features of participants (Table1).

HPV DNA was detected in seven (6.1%) cases. Five of them were HPV-18 positive while HPV-6 and HPV-66 were detected each in one participant.

Seventeen (14.9%) of participants were cigarette smokers and 95(83.3%) were not.

The smoking history in two subjects could not be identified. As the number of smokers in participants was not adequate enough for statistical analysis, we considered subjects with history of smoking more than one pack/year cigarette as smoker. In smoker individuals HPV DNA detected 1.7 times (OR=1.7) more than non-smokers, but it was not statistically significant (p=0.43).Fifteen people had history of alcohol consumption. Although HPV positivity was not statistically significant in this group (p=0.3) but odds ratio was 1.98.Oral and dental health were poor in 30 (26.3%) participants and good in 59 (51.8%) of them. The relation between oral health and HPV- positivity was not statistically significant.

Table1: Demographic characteristics and risk factors

Characteristic		Number	Percent (%)
Sex	male	52	45.6
	female	53	46.5
	unknown	9	7.89
Marital Status	married	67	58.8
	single	39	34.2
	unknown	8	7.01
Place living	urban	98	86
	rural	4	3.5
	unknown	12	10.5
Smoking	smoker	17	14.9
	nonsmoker	95	83.3
	unknown	2	1.7
Alcohol	user	15	13.1
	nonuser	96	84.2
	unknown	3	2.6
Oral Hygiene	good	59	51.8
	poor	30	26.3
	unknown	25	21.9
Number of Sexual Partner	1	51	44.7
	2	3	2.6
	>2	2	1.8
	unknown	58	50.9
Oral Sex	yes	33	28.9
	no	70	61.4
	unknown	11	*9.6*

(P= 0.76) of 33 (28.9%) participants with positive oral – sex history, 12 cases were practicing it routinely and 19 cases off and on. 70(61.4%) of the subjects had never had oral-sex, and 11(9.6%) did not answer this question.HPV DNA in those who had oral sex was detected 1.09 times more than not experienced ones but it was statistically insignificant.

Fifty one (44.7%) of participants had one sexual partner. Three of them (2.6%) had two and two (1.8%) subjects had more than two partners.

Fifty-eight (50.9%) of subjects were single or did not answer this question. In this study, the relation between HPV positivity with the number of sexual partners was not statistically significant. Five cases of HPV positive cases had one sexual partner.

In our study female HPV positive cases were 2 times more than men (OR=2.04, CI 95%: 0.35-11.65).100% of HPV positive cases were from urban area. Marital status was not statistically significant in relation to HPV positively (p=0.49) (Table2).

Table2: Demographic and risk factor characteristics in relation to HPV positivity

Demographic Characteristic[b]		HPV[a] Positive n (%)	HPV Negative n (%)	P value	Odds ratio (CI: 95%)
Sex	Male	2(28.6)	50(51.0)	0.348	2.04(0.35-11.65)
	Female	4(66.7)	49(49.5)		
Marital status	Married	3(42.9)	64(64.0)	0.49	1.7
	unmarried	4(57.1)	35(35.0)		
Smoking	Smoker	2(28.6)	15(14.3)	0.28	1.7
	nonsmoker	5(71.4)	90(85.7)		
Alcohol	User	2(28.60)	13(12.5)	0.24	1.8
	nonuser	5(71.4)	91(87.5)		
Oral Hygiene	Good	5(71.4)	54(50.5)	0.55	-
	Poor	1(14.3)	29(27.1)		
Oral sex	Yes	3(42.9)	30(31.3)	0.39	1.09
	No	4(57.1)	66(68.8)		

[a] Human Papilloma Virus; [b] Exclude participant with unknown values

Discussion

Worldwide HPV prevalence of oral cavity in healthy individuals is highly variable (5,23,24). It might be due to different population, social habits, various methods for detecting HPV DNA and sampling of oral specimen (2,5,16,23,27). Recent systematic review by National Cancer Institute (NCI), showed, the prevalence of HPV was 4.5% (95% CI: 3.9-5.1) in 4070 cancer – free subjects (5), which is similar to previous studies, HPV-16 was the most frequent type. In our study the frequency of HPV in saliva samples of 114 cancer – free subjects was 6.1%. Unlike other studies HPV-16 was not detected in saliva samples, instead HPV-18 was the most frequent type. Sahebjamee et al., in a case – control study in Tehran-Iran, showed that 25% of 20 healthy control group and 40.9% of the patients, in that study HPV -16 was the most prevalent type (27). Another study in North West of Iran, demonstrated the frequency of HPV in 74 women with cervical cancer is 62% and the most frequent type was HPV-16 (28), it is also postulated in the other study in Iran by Mortazavi et al. (19). Even though our findings were inconsistent with most conducted studies worldwide but there are some other studies in the literature that, have shown the similar results as we did. Terai et al., showed HPV-18 was the most frequent type in oral mucosa of 30 healthy individual (29). It has been demonstrated in other studies also (30).This discrepancy might be due to variety in socioeconomic and ethnic in different societies. Our study showed women are more susceptible to HPV infection compared to men. Some investigators revealed HPV prevalence is equal in men and women and in some others the male prevalence was dominant (10, 24), cumulative, still there is not any consensus about this issue.

Association between oral HPV infection and age was not statistically significant in our study.Several authors have documented the prevalence of HPV decreasing with increasing of age in cervical specimen in women (31,32). In one study odds of oral HPV infection has in-

creased with increasing of age (24), but totally its impact on frequency of oral HPV has not been investigated clearly by our knowledge yet.

We showed cigarette smoking and alcohol consumption increase the risk of HPV positivity, even though it was not statistically significant; that might be due to small number of smokers or alcohol consumers in our study.Although smoking has synergistically effect with HPV in cervical cancer (11,33),this impact has not been obviously determined in oral HPV infection. There is more controversy and conflicting data about the role of tobacco smoking on oral HPV positivity in the literature (2). For instance one study showed that smoking might impair antibody response in HPV 16/18 infected young women (34,35).Doctor Park's showed "that tobacco smoking and drinking alcohol help promote HPV invasion"(3). Therefore more studies should be done for describing smoking impact on oral HPV infection. The role of alcohol consumption also is not clearly defined in contrast to cervical cancer in women (2).

According to our study data, oral sex increases the risk of oral HPV infection, even though it was statistically insignificant. This result is consistent with other investigators (5-7,10,14,15,22). In fact some believe that the reason of rising frequency of HPV related HNSCC is related to increasing high risk sexual behaviour (6,8,12-14). We did not find any relation between number of sexual partner and oral HPV. It probably is due to small number of subjects who had more than one sexual partner.

Our study did not reveal any relation of oral health status and oral HPV infection, since many participants had not been visited by the dentist. Reviewing the literature

the link between oral hygiene and HPV positivity in oral mucosa has not been documented, whereas poor oral health may be a risk factor for unrelated HPV head and neck cancer (12,36), even though, this relation has not been revealed in HPV related HNSCC.

We showed the prevalence of HPV infection in our cancer free community is more or less like the other developing countries worldwide, stressing that HPV 18 is dominant type which could play a role in HPV related HNSCC.

It looks out most important for drawing our health care system attention to the fact that in case of decision making plans for vaccination strategy we need larger studies to find out if HPV 18 is the most dominant one to be covered.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc) have been completely observed by the authors.

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