Human Papilloma Virus in Head and Neck Squamous Cell Cancer

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Abstract

Background: Epidemiologic and molecular evidences have established a strong link between high risk types of Human Papilloma Virus and a subgroup of Head and Neck Squamous Cell Carcinomas (HNSCC). We evaluated the frequency of HPV positivity in HNSCC and its relationship to demographic and some risk factor variables in an open case-control study.

Methods: Fourteen recently diagnosed patients with squamous cell cancer of oropharynx, hypopharynx and larynx aged 18-50 years were examined from 2008-2010 in Tabriz, Iran. HPV DNA was extracted from paraffin-embedded blocks of each patient's sample for PCR evaluation. Saliva samples of 94 control cancer-free subjects were collected for DNA analysis. Multivariable logistic regression method was used to calculate odds ratio for case-control comparisons.

Results: High risk HPV was detected in 6(42.8%) patients, and 6(5.3%) control subjects which was statistically significant ($p<0.0001$). HPV-18 was the most frequent type both in the cases and controls. HPV-16 DNA was detected in two patients of the case group, but it was not detected in any of the controls. The relation between demographic and risk factor variables was not statistically significant.

Conclusion: HPV infection has a significant impact on HNSCC. Despite HPV-16 stronger impact, HPV-18 is more likely to cause malignant degeneration in such cancers amongst some communities. It is vital to introduce and conduct immunization schedules in health care systems to protect communities to some extent.

Keywords: Head and Neck Neoplasms; Squamous Cell Carcinoma; Human Papilloma Virus 16; Human Papilloma Virus 18; Sex

Please cite this article as: Asvadi Kermani I, Seifi SH, Dolatkhah R, Asvadi Kermani A, Sakhinia E, Dastgiri S, Ebrahimi A, Lotfy A, Esmaeili HA, Mohammadi G, Naderpour M, Hajalipour SH, Asghari Haggi A, Nadri M. Human Papilloma Virus in Head and Neck Squamous Cell Cancer.Iran J Cancer Prev. 2012; 5(1): 21-6.

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Received: 2 Nov. 2011
Accepted: 3 Dec. 2011
Iran J Cancer Prev 2012; 1:21-26

Introduction

Head and neck cancer is the fifth [1] most common cancer in the world. Squamous cell carcinoma includes 90% of head and neck cancers [2]. Among known risk factors, smoking and alcohol consumption are considered as two major risk factors [2-5]. However, some patients do not have any obvious risk factors. In recent years, both epidemiologic and molecular evidences have established a strong link between HPV, a highly transmitted sexually virus, and the upper aerodigestive tract cancers [2, 5-8]. This association is stronger with a subset of Head and Neck Squamous Cell Carcinoma (HNSCC), particularly oropharyngeal squamous cell carcinoma [2, 9-11]. Similar to cervical cancer in women, among more than 100 types of HPV, only high risk ones play a role in developing HNSCC [5, 12-16]. HPV-16 and HPV-18 are the two most common high risk types in this group of HNSCC respectively [5, 7, 8]. Patients with HPV-related HNSCC are usually younger, nonsmoker and non-alcoholic consumers [3, 9-13]. Therefore, it seems these tumors are different in several aspects including: molecular implication, pathological characteristics, clinical course, and response to chemoradiotherapy and outcome [6, 17,

18]. Studies conducted on molecular pathogenesis in HPV related tumors have postulated down regulation of p53 wild type and Rb protein and up regulation of P16 that is in contrary to HPV negative tumors [16, 19-21]. E6 and E7 oncogenes of HPV inactivate P53 and PRb respectively [1, 6, 10-15]. All of these evidences suggest that HPV positive head and neck squamous cell cancers are distinct entities compared to non HPV -related HNSCC [5, 6, 17]. Epidemiologic studies have indicated a decreasing prevalence of head and neck cancers while the incidence of oropharyngeal SCC is increasing [3, 20, 22]. As sexual contact and oral sex play roles in transmission of oral HPV infection, investigators believe that possible cause of increase in the frequency of HPV positive tumors is having either oral or vaginal sex partners [1-3, 23]. Considering the role of HPV infection in developing and increasing incidence of HPV-related HNSCC, it is crucial to design preventive methods through vaccination against infection, similar to cervical cancer. We conducted a current open case-control study to determine prevalence of HPV positive oropharyngeal SCC and other subsite of head and neck. Meanwhile, we evaluated the relation between demographic and risk factor variables and HPV positivity.

Materials and Methods

This open case-control study was carried out in Haematology and Oncology Research Center of Tabriz University of Medical Sciences with cooperation of Otolaryngology Department. Eligibility criteria included recently diagnosed 18-50 year old patients with histologically confirmed squamous cell cancer of oral cavity, oropharynx, hypopharynx and larynx. Informed consent was obtained from all patients. Exclusion criteria were the ones with the history of other malignancies including nasopharynx SCC. The control group included 94 people. Age and sex were matched with cancer-free subjects who were referred to the hospital for a routine hematologic check up. The study protocol was approved by ethics committee of the University.

Data collection

After informed consent was obtained from the participants, risk factor questionnaires containing information on demographic characteristics, oral hygiene, sexual behaviour, smoking and alcohol consumption were completed by the research team for all patients. Site and stage of tumor for each patient were determined and registered in the questionnaires.

The control group completed a self-administered risk factor questionnaire which contained information similar to the case group. Then, saliva samples of the controls were collected and sent to the laboratory for DNA analysis by PCR (Polymerase Chain Reaction).

For the case group, formalin-fixed, paraffin-embedded tumor samples were obtained. After revising the confirmed histology report, three to four 4-micron-thick sections of paraffin-embedded blocked of each tumor sample were sent to the molecular laboratory for DNA extraction.

Laboratory Method

DNA was extracted using salting out method; and to control DNA quality, the GAPDH gene was amplified in all samples.PCR was performed in a final reaction volume of 25 μL, containing 10 μL of template DNA, 2.5 μL 10X PCR buffer, 2 μL MgCl2 (50 mM), 1 μL dNTPs [100 μM], 3 μL of My09 Primer (10μM), 3 μL My11 Primer (10μM) and 0.5 μL of Taq DNA Polymerase (5 U/μL). The PCR conditions were as follows: preheating for 5 min at 94°C followed by 40 cycles of 1 min at 94°C, 1 min at 55°C and 1 min at 72°C and a final extension of 7 min at 72°C. 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 μL, containing 10 μL of the first reaction , 6 μL 10X PCR buffer,3 μL MgCl2 (50 mM), 1 μL dNTPs [100 μM], 6 μL Primer GP5+ (10μM), 6 μL Primer GP6+ (10μM)and 0.5 μL of Taq DNA Polymerase (5 U/μL).The PCR conditions were as follows: preheating for 4 min at 94°C, 2 min at 40 °C and 2 min at 72 °C followed by 43 cycles of 1 min at 94°C, 2 min at 40°C and 1 min and 2 min at 72°C which was followed by 1 min at 94°C, 2 min at 40 °C and 4 min at 72 °C. Amplification products obtained from HPV- positive cases were subjected to direct sequencing. Finally for genotyping of HPV, a complementary analysis of sequences obtained from blast was performed.

Statistical Analysis

Total tobacco consumption was measured as a pack-year, smoking 20 cigarettes in a year. All the participants smoked tobacco, and did not use any other substances. Classified levels for smoking were considered as: never, <1-pack year, and >1-pack year smoker. Alcohol consumption was defined as the average number of drinks per week for more than a year. We used descriptive statistics, chi-square test and odds-ratio with confidence interval of 95% for

data analysis. Association was considered to be statistically significant for a two sided P<0.05.

Results

Fourteen newly diagnosed patients with squamous cell carcinoma of oral cavity, oropharynx, hypopharynx and larynx were enrolled in an open case-control study from 2008-2010 in the Haematology and Oncology Research Center of Tabriz University of Medical Sciences. Control group included 94 cancer free subjects who were sex and age matched with the patients. Mean age of patients was 39.71±6.7 years with a range between 27-50 years. The mean for control group was 32.91±9.14, with age range of 18-50 years. Female patients were 57.1% and male patients were 42.9%. The control group consisted of 43(45.7%) males and 49(52.1%) females. HPV DNA was detected in paraffin-embedded block of tumor tissue of 8(57.1%) patients; of which, 6(42.9%) were high risk types, HPV-18 and HPV-16. HPV-18 was the most frequent type in 4(28.6%) patients and HPV-16 DNA was detected in two (14.3%) cases. HPV-6 (low risk type) was identified in other two patients. The frequency of HR-HPV in saliva samples of the control group was 4.3% in four persons which were all HPV-18. HPV-16 was not detected in any control persons. HPV-6 was detected in one (1.1%) subject. The relation of HPV positivity in case and control groups was statistically significant (p<0.0001, OR 19.55 CI 95%, 5.1-74-92).

The mean age of patients with positive HR-HPV was 42.17±5.03 years ranging from 35 to 50.

The frequency of tumor site in relation to HPV positivity is presented in Table 1.

Nine of fourteen patients were in stage IV of the disease, three in stage III and two in stage II.

HPV DNA was detected in four patients with tumor stage of IV, one in stage III, and one in stage II. For statistical analysis, tumor stages were classified in two groups: low (stages I and II), and high (stages III and IV). Association of tumor stage with HPV positivity was not statistically significant (p=0.95).

The relation of all risk factors (cigarette smoking, alcohol consumption, high risk sexual behaviour, oral health) with HPV positivity was not statistically significant. Detailed data are presented in Table 2.

Discussion

The HPV prevalence in HNSCC vary in different studies based on ethnogeographic area, sample size, method of detecting HPV DNA in tissue ,and classification of head and neck subsites [1,21,24]. In a meta-analysis, it has been reported that the prevalence of HPV related HNSCC has a wide range between 0-100% in small studies and 1.4-48.8% in large ones[1]. For instance, in South Africa its prevalence is low but in Japan is high. However, the result of another study conducted in Japan showed a low prevalence of oral HPV related to Oral Squamous Cell Carcinoma (OSCC) in normal population [25]. In another recent meta-analysis carried out by Dayanni et al. the frequency of HPV related tumor in 5681 patients was 21.95% and in oropharyngeal SCC was 41%.These results are nearly similar to previous studies [21]. As a whole, the prevalence of HPV-related HNSCC is approximately 25.9% [2, 4]. In addition, HPV prevalence in OSCC including base of tongue and tonsils is higher compared to other subsites of head and neck [2-6]. In our study, HR-HPV DNA was detected in 43.5% of patients with age range of 27-50 years, considering the fact that we excluded older patients and nasopharynx SCC. In another case-control study conducted by Sahebjamee et al. Iran, Tehran, the HPV prevalence in 20 patients was reported 40.9% [26]. However, in another study by Faleki et al. in Mashhad, HPV DNA was not detected in 21 young patients with oral squamous cell carcinoma [27]. Haratian et al. reported a 62.5% HPV frequency for 15 patients with HNSCC [28]. Based on the results obtained from studies carried out in Iran, prevalence of HPV related HNSCC is higher compared with developed countries. However, further studies are required to consolidate above-mentioned conclusion. Our study indicates a strong link between HPV and HNSCC in light of low prevalence (4.3%) of HR-HPV infection in control group.

According to many studies, HPV-16 is the most common high risk HPV in HPV related HNSCC [2, 4-7, 29]. However, the results of few studies have indicated that other HR-HPV types can be the commonest. For instance, Rensburg et al. showed HPV-18 was the only detected type among 66 patients with HPV positive tumors [30]. In addition, in another study in South Africa, HPV-18 was the only HPV type detected in 59 patients [24]. Kojima et al. found that HPV-38 is more common than the other types in Japan [31].Consistent with those studies, we found that HPV-18 is the most prevalent type of HR-HPV both in the tumor tissue and saliva samples of controls. The results, however, are inconsistent with other studies carried out in Iran on the prevalence of high risk type HPV in HNSCC and cervical cancers in women where HPV-16 had been the most frequent type [22, 26, 28]. Kreimer et al.

Table 1. Tumor site frequency in relation to HPV positivity

Tumor site	HR[1]-HPV[2] positive n (%)	HR-HPV negative n(%)	Total 14
Oral cavity	2(40)	3(60)	5
Hypopharynx	2(66.7)	1(33.3)	3
Oropharynx	0(0)	2(100)	2
Larynx	2(50)	2(50)	4

1. HR: High Risk
2. HPV: Human Papilloma Virus

Table 2. Demographic and risk factor variables relation to HPV positivity

Demographic characteristics		Patients N=14	Controls N=94	P
		n (%)		
Sex	Female	8(57.1)	49(52.1)	-
	Male	6(42.9)	43(45.7)	
	Missing	_	2(2.1)	
Smoking	Smoker	3(21.4)	14(14.9)	0.460
	Nonsmoker	11(78.6)	80(85.1)	
Alcohol	User	1(7.1)	10(10.6)	0.99
	Nonuser	13(92.9)	84(89.4)	
Oral Sex	Never	10(71.4)	67(71.3)	0.99
	Ever	4(28.6)	27(28.7)	
Oral hygiene	Good	5(35.7)	50(53.2)	0.262
	Poor	9(64.3))	44(46.8)	
Number of sexual partner	1	12	91(96.8)	0.065
	>1	2(14.3)	3(3.2)	

reported the frequency of HPV-18 to be low in oropharyngeal SCC compared to oral cavity and laryngeal SCC [4].In our study, we had only two patients with oropharyngeal SCC and this could be the reason for the higher prevalence of HPV-18 in our study. On the other hand, HPV-18 was the most frequent type in the control group as well. Other contributory factors affecting our results such as different nature of North West Iran, using different techniques for PCR and DNA sequencing should be considered. To achieve a concrete conclusion conducting further studies with larger sample size are recommended.

Some studies have shown the relevance of HPV positive HNSCC and more advanced disease and nodal metastases [2, 3, 9]. In our study, although the majority of patients had advanced stage of the disease (stage III and IV), this relation with HPV positivity was not significant due to small sample size.

Although tobacco smoking and alcohol consumption are main risk factors for HNSCC, numerous studies have demonstrated that HPV-related HNSCC patients are less likely to smoke and consume alcohol [2, 3, 16]. There is some controversy over the impact of smoking and alcohol consumption on HPV infection in HNSCC [32-36].This is contrary to cervical cancer in women where smoking has an obvious synergistical effect with HPV. Some studies have suggested additive effects of smoking on HPV positive oral cavity and oropharyngeal SCC. Other investigators have shown that HPV seropositive smokers have a higher risk for developing HNSCC [34, 37, 38]. Smith et al. reported that tobacco and alcohol have synergistical effect on positive HR-HPV [11]. Dr. Park's showed that tobacco smoking and drinking alcohol help promote HPV invasion [39].Considering the small sample size; we did not find any link between cigarette smoking and alcohol consumption and HPV positive tumor. Overall, further studies are needed to determine the impact of smoking and alcohol on HPV related HNSCC.

Although poor oral hygiene may be considered a risk factor for HNSCC [14, 17, 40], no association between HPV related HNSCC has been defined in the literature [17]. In our study, we did not find this relation either.

Accumulating data have revealed high risk sexual behaviour including oral sex and multiple sexual

partners to be the risk factors for HPV related HNSCC [20, 23, 41].In our work, this relation was not shown properly due to few patients having oral sex or more than one sexual partner. It should be mentioned that some patients had social limitations to talk about their sexual practices. Another limitation in our study included small sample size. As HNSCC, except for nasopharynx SCC, within the age range of 18-50 years was too rare in our hospital, it is difficult to comment on demographic and risk factor variables in association with HPV DNA detection.

Conclusion

HR-HPV has a definite impact on HNSCC. Given that HPV-18 is the most prevalent type in this study, the need to design and introduce a preventive program through vaccination seems more logical. Further studies are required to identify risk factors related to HPV infection.

Acknowledgement

We would like to thank the Research Deputy of Tabriz University of Medical Sciences for financial support of this project.

Conflict of Interest

There is no conflict of interest in this study.

Authors' Contribution

IAK designed the study, ShS collected the data and wrote the article, RD and AAK collected the data and revised the manuscript , ES , AE , AAH and MN contributed to the laboratory work , SD contributed to the analysis and interpretion of the data , AL, GM, MN and ShH contributed to patients diagnosis , HAE contributed to pathologic diagnosis. All authors read and approved the final revision.

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