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POLYMERASE CHAIN REACTION;

DETECTION OF HUMAN PAPILLOMA VIRUS (HPV) TYPE- 16 AND -18 INFECTION IN LARYNGEAL AND ORAL SQUAMOUS CELL CARCINOMA IN SOUTHERN IRAN.

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ABSTRACT... Khademi@yahoo.com. One fifth of cancers world wide are associated with viral infection. Epidemiologic and biomolecular evidence suggested that Human Papilloma Virus (HPV) infection may be associated with the development of head and neck cancer. **Objectives:** (1) To clarify the role of HPV infection in head and neck cancers. (2) To evaluate the presence of HPV DNA in laryngeal and oral squamous cell carcinoma in southern Iran and comparison of results with studies in other regions. **Setting:** Department of Otolaryngology-Head and Neck Surgery, Khallili Hospital, Shiraz Medical University Iran **Period:** From 2003 to 2006. **Material & Methods:** Eighty three (83) patients with Squamous Cell Carcinoma (SCC) of the larynx, 40 patients with benign mucosal lesion of the larynx (control), 47 patients with SCC of oral cavity and 10 patients with benign oral lesion were studied for the presence of HPV DNA by Polymerase Chain Reaction (PCR). **Results:** None of the laryngeal SCCs or control group was positive for HPV DNA. Only 3/47 specimens from oral SCC were positive for HPV DNA. Oral control group was negative for HPV DNA. **Conclusions:** The present work suggests that HPV infection has not important role in carcinogenesis of laryngeal or oral SCC in southern Iran. However a multi center case-control study is needed to clarify this association.

Key words: Human Papilloma Virus, Squamous Cell Carcinoma, Oral Cavity, Larynx

INTRODUCTION

An association between the presence of human papilloma virus (HPV) and the development of head and neck cancer has been established recently. The association is strengthened by the fact that the same oncogenic HPV types detected in cervical carcinomas have been identified in head and neck cancers. 22% of oral carcinomas were reported to contain HPV by any of the detection techniques. HPV 16 is the most prevalent HPV type found in oral squamous cell carcinoma. The

association of HPV with laryngeal carcinoma was first suggested by detecting typical cytopathic effects of HPV in these lesions[1].

At the moment, the evidence linking HPV to laryngeal carcinoma must be considered incomplete[1,2]. Based on the information in the literatures, it seems highly probable that the high- risk HPV 16 (and to lesser extent, HPV 18,31, and 33) are implicated in the etiology of at least a subset of laryngeal carcinomas[1].

The purpose of this study was not to evaluate the presence of HPV DNA in laryngeal and oral squamous cell carcinoma in southern Iran and comparison of results with studies in other regions.

MATERIALS AND METHODS

All newly-diagnosed patients with primary squamous cell carcinoma of the larynx and oral cavity were operated in Khallili Hospital. Department of Otolaryngology-Head and Neck Surgery, Shiraz Medical University between 2003 and 2006 were eligible for enrollment. 83 patients with SCC of the larynx and 47 patients with SCC of the oral cavity participated in the study. For control group we use from 40 patients with benign mucosal lesion of the larynx and 10 patients with benign mucosal lesion of the oral cavity. Staging of the cancerous patients was based on the AJCC criteria. Data collected from the patients was about the age, history of radiation to the head and neck, tobacco and alcohol use.

Laboratory Methods

The presence of HPV DNA in all samples was analyzed by PCR. Briefly, 10×5 µm thick sections were cut, placed in an eppendorf tube, dewaxed and then treated with proteinase k. Samples supernates were then analyzed for HPV DNA. Primer sequence for common HPV, HPV-16 and HPV-18 are shown as follow.

Beta action gene was used as internal control.
β-Actin -1
5'- ATC ATG TTT GAG ACC TTC AA 3'
β-Actin-2
5'- CAT CTC TTG CTC GAA GTC CA 3'

317bp.

PCR reactions were performed in a DNA thermal cycler. A positive control and negative control was also amplified after amplification, reaction mixture was electrophoresed by ethidium bromide staining.

Primary sequence		Size of PCR product
Common HPV	5'TTT GTT ACT GTG GTA GAT Ac 5'GAA AAA TAA ACT GTA AAT CA	140bp
HPV 16	TCA AAA GCC ACT GTC TCC TG CGT GTT CTT GAT GAT CTG CA	120BP
HPV18	GAC ACA TTG GAA AAA CTA AC TAG TGC CCA GCT ATC TTG TG	140bp

RESULTS

None of the laryngeal SCC samples or control group were positive for HPV DNA only 3 specimens from oral SCC were positive for HPV DNA. None of the control group for oral cavity was positive for HPV DNA. Demographic characteristics of four groups are shown in table I and II.

Three positive specimens are 1- 65-years old male with SCC of the tongue in TNM staging II: $T_2N_0M_0$ with history of smoking and negative history alcohol use or previous radiation to the head and neck. Pathology report was well differentiated SCC. PCR result was HPV-16 positivity.

2-76-years old female with moderately differentiated SCC of the tongue in $T_1N_2M_0$ TNM staging without history of smoking, alcohol use or previous radiation to the head and neck. PCR result was HPV-16 positivity. 3- 34-years old male with moderately differentiated SCC of the tongue in TNM staging $T_2N_0M_0$ with history of smoking and alcohol use and no previous radiation to the head and neck. PCR result was positive for common HPV DNA but result for HPV-16 and -18 DNA was negative.

Table-I. Demographic characteristics and risk factors for laryngeal squamous cell carcinoma and controls.

Characteristic	No. of case patients (%) (N=83)	No. of control subjects (%) (N=40)
Age (No. mean)	83(61.55)	40(33.00)
Sex Male Female	 82(98.7) 1(1.3)	 12(30.3) 23(57.5)
History of Radiation to the head & neck Yes No	 7(8.433) 76(91.56)	 0(0) 40(100)
Smoking Yes No	 70(84.33) 13(15.66)	 15(37.5) 25(62.5)
Alcohol use Yes No	 19(22.89) 64(77.10)	 5(12.5) 35(87.5)
Anatomic site Supraglott Glott Subglott	 18(21%) 63(75.9%) 2(2.40)	
Lymph node involvement No N1 N2 N3	 36(43.37) 29(34.93) 12(14.45) 6(7.22)	
TNM staying		
I II III IV	8(9.63) 13(15.66) 16(19.27) 55.42	
Grading Well differentiated moderately differentiated scc poorly differentiated scc	 54(65.06) 25(30.120) 4(4.81)	
Pathology Vocal cord polyp Vocal card nodule		 10(25) 30(75)
HPV DNA + -	 0.0 83(100)	 0.0 40(100)

Table-II. Demographic characteristic and risk factors for oral squamous cell carcinoma and controls

Characteristic	No. of case patients (%) (N=47)	No. of control subjects (%) (N=10)
Age (No. mean)	(59.51)	(42.2)
Sex Male Female	 24(51.1) 23(48.9)	 3(30) 7(70)
History of Radiation to the head & neck Yes No	 0(0.0) 47(100)	 0(0.0) 10(0.0.)
Smoking Yes No	 24(51.1) 23(48.9)	 2(20) 8(80)
Alcohol use Yes No	 41(87.2) 6(12.8)	 0(0) 10(100)
Anatomic site of Cancer Tongue Floor of mouth Gingival Buccal mucosa Lip	 39(83) 2(4.3) 2(4.3) 2(4.3) 2(4.3)	
Lymph node involvement No N1 N2 N3	 24(43.6) 7(12.7) 10(18.2) 6(10.9)	
TNM staying I II III IV	 17(36.2) 5(10.6) 7(14.9) 18(38.3)	
Grading Well differentiated scc moderately differentiated scc poorly differentiated scc	 31(56.4) 13(23.6) 3(5.5)	
Pathology Mucosus retention cyst Pyogenic granoloma Fibroma		 4(40 2(20) 4(40)
HPV DNA + -	 3(6.4) 44(93.6)	 0 10(100)

DISCUSSION

The association of HPV with laryngeal carcinoma was first suggested by detecting typical cytopathic effects of HPV in these lesions by Syrjänen and Syrjänen in 1981[1].

The most convincing evidence to implicate HPV to laryngeal cancer is derived from the studies demonstrating HPV DNA in the cancer lesion by different hybridization techniques and PCR by Syrjänen, 1997; Kashima HK et al; 1997; Herrero, 2003[1,3,4].

Looking at laryngeal carcinoma by Brandsma et al; Garcia- Milian et al and Nishioka et al, the rate of infection in control samples was approximately 5%[5-6-7]. There in a large variation in the reported prevalence, with HPV positivity varying from 3 to 47% in case-control studies and 0 to 100% in case series[1]. HPV-16 is the predominant subtype. Although most laryngeal carcinomas are squamous cell in origin (>90%), the geographic variation in incidence and sub-site, suggests considerable heterogeneity in etiology.

The overwhelming relative risks associated with tobacco smoking and alcohol consumption in practice make the evaluation of other risk factors difficult. Women with primary invasive cervical cancer had a significantly increased relative risk of 3/4 for subsequent laryngeal carcinoma[2]. There are few studies about sexual behavior of patients with HPV-Positive laryngeal and oral squamous cell carcinoma which may be a mechanism by which HPV is introduced into the oral cavity and larynx.

In review of all studies, two major problems are identified. First, there is much heterogeneity in the methods used to collect specimens and the sites from which they are isolated. Second, the techniques used to isolate viral DNA vary considerably, both in sensitivity and the ability to identify viral genome. In particular, it is impossible to know whether the extent of variation in HPV prevalence between studies represents a fundamental diversity between different populations or whether it simply reflects the discrepancy in methods[8]. To confirm the association in epidemiologic terms, a suitable powered, multi center, case-control study is needed with the ability to examine the difference in laryngeal sub-sites and relation with smoking and other risk factors. Stina syrjanen was the first to provide in 1987[1].

Although PCR is the most sensitive method in detecting HPV infection it still produces varied results, with prevalence rates of HPV infection varying from 0% to 100%. The difference may be due to two factors. First, there could be inherent differences in the populations being studied. Second, the choice of primers used in the PCR could affect HPV detection. For this, a large-scale multi-center case-control study with adequate statistical power is need to prove the association between HPV infection and oral squamous cell carcinoma[1].

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