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Research Article

Prevalence of Human Papilloma Virus infection in nonsmoker patients with Squamous Cell carcinoma of tongue by polymerase Chain Reaction in south of Iran, Shiraz

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Abstract: Human papilloma virus(HPV) as one of the important causal agent of gynecological cancers appears to play an important role in cancer of oral cavity and oropharynx. This study was aimed to evaluate the relationship between presence of HPV and squamous cell carcinoma(SCC) of tongue in non-smokers patients in south of Iran. In this case control study, pathologic specimen of 35 nonsmoker patients with histological diagnosis of tongue SCC, compared with 30 specimen of patient with tongue lesion other than intraepithelial neoplasms. Polymerase chain reaction (PCR) was used to detect HPV16 and 18 genome in both groups.(10) of (35) in case group and (1) of (30) in control group, 28.5% versus3.3% showed presence of HPV genome by use of PCR. chi-square test used for statistical analysis of data (p value=0.007)**.** Although this study support the strong association between HPV infection and tongue SCC in non-smokers, but because of rarity of tongue SCC, planning for more advanced studies are suggested to prove this association.
Keywords: Human Papilloma Virus, Squamous Cell Carcinoma, Polymerase chain reaction, Tongue cancer.

INTRODUCTION

Human papilloma virus (HPV) genus belongs to papilloma virus genus of the papova viridae family. It has more than120 identified subtypes [1].

Presence of HPV DNA was reported in much human cancer such as oral, esophageal, laryngeal and uterine cervix squamous cell carcinoma (SCC); so it is known one of the important carcinogens. The carcinogenic potential of the HPV is present in the genome of virus; gene product neutralize protein product of the tumor suppressor genes such as: P53, P105 and RB [1].

The presence of HPV as a single risk factor in cervix may increase cervical intraepithelial lesion up to116 times, [2] So WHO considers HPV as human carcinogen [3].

Because of all cervical cancers have been associated with HPV infection [3,4]. researchers evaluating the role of HPV in cancer of head and neck. The HPV prevalence in these tumors varies broadly in different population, sub sites, type of the specimen, and detection method [3,4,6].

We know that practically speaking cancer of oral cavity mucosa, tongue, oropharynx and larynx is synonymous with squamous cell carcinoma; the major risk factors for oral cancer are smoking, alcohol consumption, micronutrient deficiencies and poor oral hygiene [5,6,7].

Nowadays oral cavity and oropharynx cancer are at the top of major worldwide public health problem [5,7]. On the basis of BNCA (Brazilian National Cancer institute) reports, the ranking incidence of oral cavity mucosa, in 2005 is the eighth most common type of cancer in women and men [7].

In this study we investigate the relation between the HPV infections in tongue SCC In the patients which referred to affiliated hospitals of Shiraz University of medical sciences during the period of 2000-2012.

MATERIAL AND METHODS

We design a case control study which conducted from April 2000 to October 2012. This project started with, selection of the most proper paraffin block of the patients with tongue SCC who referred to Shiraz medical university hospitals from

pathology archive of Nemazi, Faghihi, and khalili hospital during recent decade (2000-2012). The specimens of smoker patient excluded from the study for elimination of nicotine as a carcinogen. Then 37 and 30 paraffin block of tongue tissue of patients with SCC and without evidence of epithelial lesion was placed in case and control groups.

Initially we cut one section for H&E staining and then10 sections all 10 µm in thickness and finally another section for H&E staining, for confirmation of presence of tumoral tissue in all cut sections. All of ten sections placed in sterile Eppndorf tube for subsequent DNA extraction. It is worthy of special note that all of the surfaces and instruments before and after the next sampling were cleared by xylol and then by one normal HCL.

The presence of HPV DNA in all samples was analyzed by PCR. Briefly, 10×10 µm thick sections were cut, placed in an Eppendorf tube, de waxed 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 actin gene was used as internal control.

B-Actin -1
5'- ATC ATG TTT GAG ACC TTC AA 3'
B-Actin-2
5'- CAT CTC TTG CTC GAA GT 317bp.

PCR reactions were performed in a DNA thermal cycler (Mastercycler Gradient Eppendorf ;Germany).

A positive control and negative control was also amplified.

Use of the positive and negative control in each electrophoresis plate confirmed the accuracy of the method. The positive control was taken from HPV infected skin, and the negative control by water replacement for extracted DNA. After amplification, reaction mixture was electrophoresed and stained with Ethidium Bromide.

Table-1: Primer sequences of HPV types for PCR

HPV type	Primer sequence	Band size
Common type	5'TTT GTT ACT GTG GTA GAT Ac 5'GAA AAA TAA ACT GTA AAT CA	140 kbp
HPV 16	TCA AAA GCC ACT GTC TCC TG CGT GTT CTT GAT GAT CTG CA	120kbp
HPV 18	GAC ACA TTG GAA AAA CTA AC TAG TGC CCA GCT ATC TTG TG	140kbp

RESULT

Our final case and control group consisted of 35 and 30 specimen..

We review all of the H&E stained slides in case and control group; In the case group 18, 14, and3 cases showed well, moderately, and poorly differentiated SCC respectively.

The age range was 17 to 76 years old. The mean age was 57 years old. Only 3 cases were younger than 40 years old. The male to female ratio was 60% to 40%. HPV DNA amplified by PCR test, There was: 10(28.5%) and only one (3.3%) positive result in case and control groups respectively. All positive results were older than 40 years old and 6 of 10 in case and zero in the control group were positive for HPV16 , no positive results was seen for HPV 18.four case of the positive result for HPV 16 was related to well differentiated SCC, and the remainder was moderately differentiated SCC. *Chi-square* test used to analyze the PCR results. There was a significant difference between these two groups; So HPV infection especially with high risk HPV type 16 may play an important role in tongue SCC in our study population (p value=0.007).

DISCUSSION

Several researchers studied the relation between HPV infection and head and neck tumors. They obtained broadly different results, but most of them concluded that HPV infection especially oncogenic type of 16 and 18 has been played a significant role in tumors of these sites.

Wei Li et al compared tonsillar tumors between Australian and Chinese population; They found significant difference in the HPV presence in these two groups 0 of 16 in Chinese specimen [10]. to the best of our review articles with low prevalence of HPV reports have been worked with paraffin embedded tissue, like our study; And the positive results ranges between 8.4% to 42% [10-14].

These discrepancies of the results as mentioned in some articles may be because of the use of fresh tissue in high prevalence of HPV reports and use of paraffin embedded tissue in low prevalence reports of the HPV positivity. Series of factors such as formalin fixation, paraffin embedding, deparaffinization, and detection methods can damage the DNA integrity and lowers the detection rate [14].

CONCLUSION

HPV DNA presence in case and control groups differed significantly (p value=0.007).So this study let's us to concluded that HPV is an important risk factors in tongue SCC carcinogenesis.

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