The Iranian Journal of
Otorhinolaryngology
Vol.18, No.43, Spring-2006

مجله گوش، گلو و بینی و حنجره ایران
سال هجدهم، شماره چهل و سوم، بهار ۱۳۸۵

Study on the association between squamous cell carcinoma of larynx and HPV by PCR

Sharifi N. MD[1], Omidi A. MD[2], Tavakkol Afshari J. MD[3], Ghannadan A. MD[4], Khodadust [5]

[1]Assistant Professor of Pathology, [2]Professor of Pathology, [3]Assistant Professor Immunology, [4]Resident of Pathology, [5]Biologist

Abstract

Introduction: Abroad epidemiologic studies have suggested human papillomavirus (HPV) to be an etiological agent in Squamous cell carcinoma (SCC) of larynx. In this study we investigated the frequency of HPV 16 infection in various neoplastic and non-neoplastic laryngeal tissue.

Materials and Methods: The study was done on 55 cases of laryngeal SCC, 10 cases of dysplastic and 5 cases of normal tissues were analyzed by polymerase chain reaction (PCR) for the presence of HPV 16.

Results: None of the five normal and ten dysplastic laryngeal tissues showed the presence of HPV. Seven of fifty-five Squamous cell carcinoma of larynx (12.7%) were positive for HPV type 16.

Conclusions: The results suggested that HPV DNA has a major role in squamous cell carcinoma of larynx

Keywords: HPV, Squamous cell carcinoma, Larynx

Introduction

Laryngeal Squamous Cell Carcinoma (SCC) is the most frequent malignant tumor in the head and neck. Human Papilloma Virus (HPV) has been implicated in proliferative and invasive lesions of the larynx (1-9).

More than 75 different HPV genotypes have been cloned, and categorized in low and high risk types. The low risk types are associated with benign lesions and high risk HPV genotypes have marked malignant potential and are frequently associated with epithelial dysplasia and Squamous Cell Carcinoma at several anatomic sites.

The low risk HPV-6 and 11 DNA sequences were detected in approximately 100% of Laryngeal Papillomatosis (10) and the high risk HPV (16,18) have been found in high percentage of Laryngeal Verrucous Carcinoma (11).

Although almost all epithelial cancers of head and neck and genital system harbour HPV DAN and the relationship between HPV and anogenital cancers have been clearly demonstrated in numerous studies, a putative relationship between HPV and Laryngeal Carcinoma is still controversial.

Sharifi N, MD.
Address: Mashhad University of Medical Sciences
Email: N_sharify@mums.ac.ir
Acceptation date: 83/7/14 Confirmation date: 83/9/5

In 1986 HPV DNA was cloned from a laryngeal carcinoma (1) and HPV DNA has been demonstrated in carcinomas arising from preexisting laryngeal papilloma (2,4).
Although epidemiologic investigations have unquestionably established tobacco and alcohol as major risk factors for Squamous Cell Carcinoma of the larynx(2) the role of human Papilloma virus (HPV) in this tumor is still unclear. HPV is double stranded DNA virus with a circular genome of 7600-8000 base pairs that replicate only within the permissive host cells (3).
The life cycle of HPV is tightly linked to squamous cell differentiation that cause abnormal maturation and differentiation of epithelial cell (4). Hence in the present study, we analyzed HPV infection in neoplastic and non-neoplastic laryngeal tissues.

Materials and Methods

55 samples of invasive squamous cell carcinoma and 10 sample of dysplastic and 5 samples of normal laryngeal tissues were included in this study. Specimens were fixed in 10 % formalin, 10-15 µm tick sections taken from paraffin embedded tissue and stained with Hematoxylin-Eosin for routine histopathologic examination.
Blocks without malignant tissue were omitted but all other specimens were selected with enough tumoral component. Sections for each block were cut for PCR. Control sections before and after sectioning for PCR were cut and stained with H&E in order to ensure that the sections for PCR did contain tumor tissue. The sections for PCR were placed in a 1.5 ml tube and 200 µl digestion buffer (10 mM Tris PH=7.0, 1mM EDTA, 200 µg/ml Proteinase K) was added. The sections were digested at 65°C for 10 min. The Samples together with appropriate positive and negative controls, were amplified with primers targeting a 315 bp fragment of the beta-actin gene in order to ensure the integrity of the DNA.
Finally PCR was performed with the universally accepted consensus primers HPF (GP65) and HPR (GP66) with some modification.
The amplified samples were evaluated by gel electrophoresis and photographed under UV transillumination.

Results

PCR amplification of HPV DNA showed that all normal and dysplastic laryngeal tissue were negative for HPV and 7 of 55 cases Squamous cell carcinoma of larynx were positive for HPV16(12.7%) Of the invasive carcinomas which examined for HPV, 29% were well differentiated, 41% were moderately differentiated, and 29% were poorly differentiated. There was no correlation between the site of the tumor and the detection of HPV. There was also no significant correlation between the presence of HPV in laryngeal carcinoma and clinicopathologic aspects such as age, site, grade, of the laryngeal carcinoma (table 1,2,3) (figure 1).

Table 1: HPV positivity laryngeal tissue

Larynx		Number		HPV PCR Positive (%)
Normal		5		0(0)
Dysphasia		10		0(0)
SCC	Grade1	55	16	7(12.72)
	Grade2		23	
	Grade 3		16	

Table 2. distribution of laryngeal SCC according to site

Site	No (%)
Supraglottic SCC	31 (56.3)
Glottic SCC	16 (29)
Infraglottic SCC	8 (14.5)

Table 3. laryngeal carcinoma (SCC) according to age and sex.

Mean of age (years)	(range) (years)	sex Male (%)	Female (%)
58	37-82	43(28.1)	12(21.8)

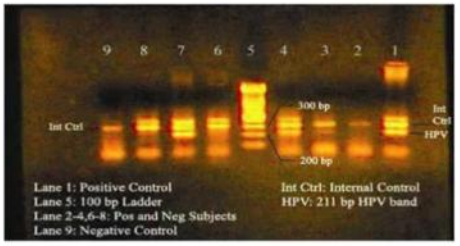

Figure 1: HPV PCR Product in Gel Electrophoresis

Discussion

Carcinogenesis is a multistep process involving genetic and epigenetic changes, which ultimately leads to activation of proto-oncogenes and or inactivation of tumor suppressor genes. This multi event nature of carcinogenesis has lead to extensive studies on oncogenes and antioncogenes, and viral factors involved in animal and human cancers. HPV has been detected by numerous investigations in squamous cell carcinoma of anogenital region (12,13).

It is unclear whether the virus is causally associated with squamous cell carcinoma of the larynx. Squamous cell carcinoma of the larynx is the single most common non-cutaneous organ to harbor a primary cancer in the head and neck (14,15). Smoking and alcohol consumption are considered highly significant etiologic factors in this disease (16,17).

Recent evidence has suggested a possible role for HPV infection and RAS oncogene activation as well (18). The range of reported frequency of HPV in this Tumor is 3-58% (Table 3).

Several Studies showed that high risk HPV 16-18 and 33 were positive in 51% of 102 laryngeal SCC in northeast of China.

Some researcher suggested that the high detection ratio of HPV in laryngeal SCC resulted from contamination (19).

In this study, however every effort was made to avoid contamination. Probably the difference in the prevalence of HPV infection may partly depends on the primers used in individual studies. The biological activities between high risk and low risk HPV mainly due to the different binding affinity of the E6 and E7 proteins of the E6 and E7 proteins to the tumor suppressor proteins P53 and Rb. The possible pathogenic role of HPV in laryngeal carcinoma has also been studied (20).

He believed that HPV 16 was the most type that infected laryngeal carcinoma and other exogenous risk factors such as Nicotine and alcohol participate their actions. Several studies showed significant correlation between presence HPV and expression of PCNA (21-23).

This fact explains that high risk HPV can inactivate P53 leading loss of its normal anti proliferative activity (24). Other studies have shown that HPV can also directly activate cellular proliferation (25,26). A study on 102 laryngeal carcinoma from China showed P53 accumulation in 58% of HPV positive tumors (21).

Conclusion

Result of the present work agree with data from the literature that under scores a relevant prevalence of HPV sequence in larynx carcinoma. However questions remain open and might find answers in larger population-based studies.

Acknowledgments

This study was supported by a grant from vice chancellor of research, Mashhad University of Medical Sciences (MUMS) and the authors thank for help.

References

1- Herrero R. Human papillomavirus and cancer of the upper aerodigestive tract. J Natl Cancer Inst Monogr 2003; (31): 47-51.

2- Fu M, Liu SB, Wang DY, Hou FL, Li QL. The detection and analysis of human papillomavirus in respiratory tract papillomas by PCR/RFLP study. Lin Chuang Er Bi Yan Hou Ke Za Zhi 2000; 14(7): 317-9.

3- Chen BB, Bao QY, Wang ZM, Jin JX, Huang SY et al . Detection of HPV-DNA and humoral immunity in juvenile laryngeal papilloma. Chuang Er Bi Yan Hou Ke Za Zhi 2000;14(6):262-4.

4- Wang L, Zhang T, Zhang F. Study on the association between squamous cell carcinoma of larynx and HPV subgene by PCR. Zhonghua Er Bi Yan Hou Ke Za Zhi 1999; 34(2):106-7.

5- Badaracco G, Venuti A, Morello R, Muller A, Marcante ML. Human papillomavirus in head and neck carcinomas: prevail physical status and relationship with clinical/ pathological parameters. Anticancer Res 2000; 20(2B): 130l-5.

6- Szentirmay Z, Szanto I, Balint I, Polus K, Remenar E et al. Causal association between human papilloma virus infection and head and neck and esophageal squamous cell carcinoma. Magy onkol 2002; 46(1): 35-41.

7- Rodrigo JP, Suarez C, Gonzalez MV. Lazo PS, Ramon S et al. Variability of genetic alterations in different sites of head and neck cancer. Laryngoscope 2001; 111(7) 1297-301.

8- Suarez PA, Adler K, Luna MA, Naggar AK, Abdul-karim FW et al. Papillary squamous cell carcinomas of the upper aerodigestive tract: a clinicopathologic and molecular study. Head and Neck 2000; 22(4): 360-8.

9- Mutiu A, Alexiu I, Chiva M, petica M, Anton G et al. Detection of human Papillomavirus gene sequences in cell lines derived from laryngeal tumors. J Cell Mol Med 2001; 5 (1): 49-59.

10-Abramson AL,Steinberg BM, Winkler B. Laryngeal papillomatosis: clinical, histopathologic and molecular studies. Laryngoscope 1987; 97(6): 678-85

11- Brandsma JL, Steinberg BM, Abramson AL, Winkler B. Presence of human papillomavirus type 16 related sequences in verrucous carcinoma of the larynx .Cancer Res 1986; 46(4):2185-8.

12- Crook T, Wrede D, Tidy JA, Mason WP, Evans DJ et al. Clonal P53 mutation in primary cervical cancer, Association with human papillomavirus-negative tumors, Lancet 1992; 339: 1070-1073.

13- Fujinaga Y, shimada M, Okazawa K, Fukushima M, Kato I. Simultaneous detection and typing of genital human papillomavirus DNA using the polymerase chain reaction. Journal of General Virology 1991; 72: 1039-1044.

14- Landis SH, Murray T, Bolden S, Wingo P. Cancer statistics. Cancer J Clin 1998; 43:7-26.

15- Muir C, Weliand L. Upper aerodigestive tract cansers. Cancer 1995; 75:147-153.

16- Plich BZ, Dorfman DM, Brodsky GL, Goodman ML. Pathology of laryngeal malignacies.In:Fried MP(ed). The larynx:a multidisciplinary approach, 2nd ed. Mosby;St Louis :1996. p. 461-485.

17- Koufman J A, Burke AJ. The etiology and pathogenesis of laryngeal carcinoma. Otolaryngol Clin North 1997; 30:1-19.

18- Nguyen C, Naghibzadeh B, Black MJ, Rochon L, shenouda G. 1996 Glottic microinvasive carcinoma: Is it different from carcinoma in situ? J Otolaryngol 1996; 25: 223-226.

19- Lindeberg H, Krogdahl A. Laryngeal cancer and human papillomavirus : HPV is absent in the majority of laryngeal carcinoma. Cancer letters 1999; 146: 9-13.

20- Arndt O, Brock G, Mullender A. The detection of HPV DNA in formalin fixed invasive squamous cell cancer of the larynx by polymerase chain reaction.Laryngorhino-otologie 1994; 73: 527-532.
21- Bravo R. Synthesis of the nuclear protein cyclin (PCNA) and its relationship with DNA replication. Exp Cell Res 1986; 163: 287-293.
22- Zidar N, Gale V, Cor A et al. Expression of Ki-67 antigen and PCNA in benign and malignant epithelial lesions of the larynx. J Laryngol Otol 1996;110: 440-445.
23- Sarac S, Ayham A, Hosal AS et al. Prognostic significance of PCNA expression in laryngeal cancer. Arch Otolaryngol Head Neck Surg 1998; 24:1321- 1324.
24 - Lakshmi S, Nair MB, Jayaprakash PG et al . P53 protein and tumorigenesis in the uterine cervix. Gen Diagn Pathol 1997; 142: 281-287.
25- Shikano K, Sasono H, Ito K et al. The correlation between HPV infection and cell proliferate activity in uterine cervical adenocarcinoma. Tohoku J Exp Med 1993;169: 279-188.
26- MA XL, Ueno K, Pan ZM et al. Human papillumavirus DNA sequences and p53 over-expression in laryngeal squamous cell carcinomas in Northeast Cina. J Med Virol 1998; 54(3):186-191.

****

خلاصه

مطالعه همراهی بین سرطان سلول سنگفرشی حنجره و ویروس HPV توسط PCR

دکتر نوریه شریفی، دکتر امیدی، دکتر توکل افشاری، دکتر قنادان، خدادوست

مقدمه : بررسی های اپیدمیولوژیک نقش اتیولوژیک ویروس پاپیلومای انسانی (HPV) را در سرطان سلول سنگفرشی حنجره مورد تأیید قرارمی دهد. در این مطالعه میزان بروز ویروس پاپیلومای تیپ ۱۶ (HPV_{16}) درکارسینوم سلول سنگفرشی و بافت نرمال حنجره مورد بررسی قرار می گیرد.

روش کار: این مطالعه در مورد ۵۵ نمونه، ۴۰ مورد کارسینوم سلول سنگفرشی حنجره، ۱۰ مورد مخاط دیسپلازیک و ۵ مورد بافت نرمال حنجره به روش مولکولی PCR جهت بررسی حضور HPV_{16} انجام شده است.

نتایج: مخاط نرمال و ۱۰ مورد بافت دیسپلازیک حنجره هیچ کدام آلودگی به ویروس HPV را نشان ندادند. ۷ مورد از ۴۰ نمونه کارسینوم سلول سنگفرشی ویروس پاپیلومای انسانی را نشان دادند(۱۲/۷٪).

نتیجه گیری: نتایج به دست آمده نقش اتیولوژیک DNA ویروس پاپیلوما را درکارسینوم سلول سنگفرشی حنجره نشان می دهد.

واژه های کلیدی: ویروس پاپیلومای انسانی (HPV)،کارسینوم سلول سنگفرشی، حنجره