ORIGINAL REPORT

Human Papilloma Virus and Esophageal Squamous Cell Carcinoma

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Received: 13 Nov. 2011; Received in revised form: 29 May 2012 ; Accepted: 4 Jan. 2013

Abstract- Human papilloma virus (HPV) has been suggested as an etiology of esophageal squamous cell carcinoma (SCC). The aim of this study was to investigate the prevalence of HPV infection in esophageal SCCs in our region with strict contamination control to prevent false positive results. Thirty cases of esophageal squamous cell carcinomas were chosen by simple random selection in a period of two years. PCR for target sequence of HPV L1 gene was performed on nucleic acid extracted from samples by means of GP5+/GP6+ primers. All tissue samples in both case and control groups were negative for HPV-DNA. Although the number of cases in this study was limited, the contribution of HPV in substantial number of esophageal SCCs in our region is unlikely.

Acta Medica Iranica, 2013; 51(4): 242-245.

Keywords: Human papilloma virus; Polymerase chain reaction; Squamous cell carcinoma

Introduction

Squamous cell carcinoma (SCC) is one of the common malignancies of esophagus (1,2). Because the tumor is usually advanced at the time of diagnosis, it causes high morbidity and mortality. Prevalence of esophageal SCC is different in distinct geographic and cultural conditions, and esophageal SCC is uncommon in United State and Western Europe in contrast to China, Latin America, Middle Asia and Iran (2,3). Genetic and environmental factors play a role in pathogenesis of esophageal SCC; multiple environmental etiologies have been suggested for esophageal SCC, for example: smoking, alcohol consumption, diet and caustic agents (4,5). Human papilloma virus (HPV) has also been suggested as an etiology (6).

Human papilloma virus (HPV) is an oncogenic, double-stranded, DNA-virus that infects skin and mucosa. Usually associated with benign papillomas, mucosal HPV Infection is one of the most common sexually transmitted infections (7). Moreover, mucosal infection with high-risk types, such as HPV 16 and 18, can rarely progress to dysplasia and cancer, a process that takes a long time to occur (7,8). Viral oncoproteins E6 and E7 target p53 and retinoblastoma protein (RB), interfere with cell cycle, and initiate malignant transformation. SCC and adenocarcinoma of uterine cervix, vagina, and vulva; and SCC of oropharynx are among the tumors caused by HPV. Oropharyngeal SCC when associated with HPV shows better prognosis in contrast to its non-HPV related counterpart (9,10). If a relation exists between HPV and SCC in esophagus, HPV vaccination can be utilized for prevention of this tumor; meanwhile, HPV detection can be valuable for determination of prognosis.

The aim of this study was to investigate the prevalence of HPV infection in esophageal SCCs in our region with strict contamination control to prevent false positive results.

Materials and Methods

In a previous study, prevalence of HPV infection in normal esophagus and esophageal SCC were about 0% (q) and 23% (p) respectively (11). According to the latter, sample size of this case control study was calculated for comparison of qualitative characteristics analysis. Consequently, thirty cases of esophageal squamous cell carcinomas were chosen by simple random selection in a period of two years from 2007 to 2009 by searching the admission database of Cancer Institute, Tehran University of Medical Sciences. Paraffin blocks containing tumor tissue were available in all cases. Thirty samples of gastroesophagectomy for gastric adenocarcinoma with involved esophageal margin were also selected by the same method as control

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group; likewise, paraffin block containing normal esophageal tissue was available in each sample. Patients are admitted to this referral center from all regions of Iran, representing a wide range of socioeconomic levels. H&E stained slide of each paraffin block was reviewed for the presence of tumor in case group and absence of tumor in control group. Demographic and pathologic data as well as PCR results of case and control groups were analyzed by SPSS version 12.0 (SPSS Inc).

Extraction

From each paraffin block, two 5 µM sections were prepared using a new disposable blade; the sections were transferred to a 1.5 ml microtube by a disposable applicator. The tubes were briefly centrifuged to make a paraffin embedded tissue pellet. Then tissue was deparafinated by xylene and nucleic acid was extracted using Roche High Pure RNA Paraffin Kit (Roche Diagnostics, Indianapolis, IN) as instructed by the manufacturer without going through the DNA elimination procedure. Eluted DNA was stored in a -20 ºC freezer until HPV detection was performed.

Polymerase chain reaction (PCR)

To ensure the validity of purification procedure and exclusion of PCR inhibition, Homo sapiens hydroxymethylbilane synthase (HMBS) was amplified as endogenous internal control as described previously (12,13).

The PCR for target sequence of HPV L1 gene was performed in 20 µl reaction containing 0.5 µM of each forward and reverse GP5+/GP6+ primers, and 10 µl SYBR Premix Ex Taq (Takara Bio, Ostu, Shiga, Japan). The PCR method for HPV L1 region was validated by means of six HPV 16 samples, proved by sequencing, from uterine cervix squamous cell carcinoma samples. The touch-down PCR was employed for its superior sensitivity (14). The PCR conditions were: initial denaturation at 94 for 0.5 min; then 45 cycles as follows: 95°C for 10 seconds; first initial 20 cycles at 60°C to 50°C for 20 seconds (with 0.5°C decrease in each cycle followed by 25 cycles of 50°C for 20 seconds; and 72°C for 34 seconds. The Real-time acquisition was performed during annealing phase on FAM channel. The touchdown annealing phase composed of 20 cycles of 60°C to 50°C with 0.5°C decrease in each cycle. Finally a 5 min terminal extension was performed followed by melting analysis of PCR product. Target melting temperature, and the sequence of primers are shown in table 1.

A rotor-gene 3000 real-time thermal-cycler (Corbett Research, Mortlake, Australia) was used for internal-control and target amplification reactions. Positive control of HPV type 16 was included in each run.

Table 1. Sequence of the primer utilized in this study.

Primer	Sequence 5′-3′	Melting Temperature (^{o}C)
GP5+/GP6+	TTTGTTACTGTGGTAGATACTAC	80
	GAAAAATAAACTGTAAATCATATTC	
HMBS	GCCTGCAGTTTGAAATCAGTG	84
	CGGGACGGGCTTTAGCTA	

Table 2. Pathological and clinical information of the case group.

		Frequency	Percentage %
Grade of tumor	Poorly Differentiated	5	16.7%
	Moderately Differentiated	15	50.0%
	Well Differentiated	10	33.3%
Site of tumor	Upper Third	7	23.3%
	Middle Third	12	40.0%
	Lower Third	11	36.7%
Lymph node involvement	Yes	12	40.0%
	No	10	33.3%
	Can not be assessed	8	26.7%
Depth of invasion	Lamina propria or submucosa	3	10.0%
	Muscularis propria	5	16.7%
	Adventitia	22	73.3%
Metastasis	Yes	5	16.7%
	Can not be assessed	25	83.3%

Results

There were 30 patients in case group, 14 of which were male and the rest were female. Average age of the patients in case group was 59.6 and ranged from 30 to 83 years old. There were 30 patients in control group, 16 of which were male and the rest were female. Average age of the patients in control group was 62 and ranged from 41 to 82 years old.

All tumors of case group were SCC with 33% grade I, 50% grade II and the rest were grade III. Tumor depth of invasion, tumor location and lymph node involvement are summarized in table 2.

All tissue samples in both groups were positive for HMBS internal control gene and negative for HPV L1 gene.

Discussion

Some authors from Iran, China, Latin America and Japan have implied a causative role for HPV in esophageal SCC (11,15-18). Meanwhile, a number of reports from other countries have questioned a substantial relation (19,20). Therefore, there is no consensus agreement on this issue. The reason might be that, not only geographic distribution of HPV infection and its genotypes are varied, but also the sensitivity and specificity of HPV detection methods are different (21,22). Secondary infection of the neoplastic squamous epithelium has also been suggested by some authors (19). Most of the studies that suggested a role for HPV in esophageal SSC were based on PCR. Being extremely sensitive method, conventional-PCR and especially nested-PCR are prone to contamination by post-PCR products that cause false positive results.

An advantage of this study was employment of real-time PCR. In real-time PCR, in contrast to conventional PCR, the estimation of the relative amount of target sequence is possible by comparing threshold cycles (Ct) of target and internal control genes. Because malignant tumors are clonal, if there is a causative virus, it is expected to be present at high amounts. In other words, the causative virus ought to be present in many tumor cell nuclei (8,23). Evidence from previous studies suggests that head and neck carcinomas have been no exception to this assumption (10). Therefore, when there are small amounts of viral DNA in a tumor, contamination or secondary infection may be more likely than a real cause-effect phenomenon (19). Moreover, in conventional PCR where products are detected after completion of the amplification phase, contamination is always a concern (24). Another advantage of the real-time PCR method is that amplification and detection are performed in the same time, and the system is closed. Therefore in the latter, the risk of PCR contamination with amplicons is minimal, which in turn decreases false positive rate. This may be the main reason behind the fact that recent reports question a causative role for HPV in esophageal carcinomas (19-21).

A limitation of this study, common to all studies that use L1 amplification, is that in minority of cases HPV integrates to host genome and parts of L1 might be deleted. Therefore, although HPV is present, it is not amplified and the test becomes false negative (13). The number of these cases are small and it is unlikely that it highly affected the results of the current study (22). Moreover, employment of formalin fixed paraffin embedded tissue is another limitation of this study. However, internal control amplifications was used to minimized the effect of formalin fixation on the results of this study.

In conclusion, although the number of cases in this study was limited, the contribution of HPV in substantial number of esophageal SCCs in our region is unlikely.

Acknowledgement

This work was supported form a grant from Tehran University of Medical Sciences Research Center.

The authors would like to thank Ms Fatemeh Ramezani and Ms Behnaz Sarhandi for their devoted support.

References

1. Devesa SS, Blot WJ, Fraumeni JF. Changing patterns in the incidence of esophageal and gastric carcinoma in the United States. Cancer 1998;83(10):2049-53.
2. Muñoz N, Day NE. Esophageal cancer. In: Schottenfeld D, Fraumeni JF Jr, editors. Cancer Epidemiology and Prevention. 2006, Oxford University Press, USA
3. Parkin DM, Pisani P, Ferlay J. Estimates of the worldwide incidence of 25 major cancers in 1990. Int J Cncer 1999;80(6):827-41.
4. Holmes RS, Vaughan TL. Epidemiology and pathogenesis of esophageal cancer. Semin Radiat Oncol 2007;17(1):2-9.
5. Valsecchi MG. Modelling the relative risk of esophageal cancer in a case-control study. J Clin Epidemiol 1992;45(4):347-55.
6. Turner JR, Shen LH, Crum CP, Dean PJ, Odze RD. Low prevalence of human papillomavirus infection in

esophageal squamous cell carcinomas from North America: analysis by a highly sensitive and specific polymerase chain reaction-based approach. Human pathology 1997;28(2):174-8.
7. Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. Nature Reviews Cancer 2002;2(5):342-50.
8. Doorbar J. The papillomavirus life cycle. J Clin Virol 2005;32 Suppl 1:S7-15..
9. Ang KK, Harris J, Wheeler R, Weber R, Rosenthal DI, Nguyen-Tân PF, Westra WH, Chung CH, Jordan RC, Lu C, Kim H, Axelrod R,Silverman CC, Redmond KP, Gillison ML. Human papillomavirus and survival of patients with oropharyngeal cancer. N Engl J Med 2010;363(1):24-35.
10. Schlencht NF. Prognostic value of human papillomavirus in the survival of head and neck cancer patients: an overview of the evidence. Oncol Rep 2005;14(5):1239-47.
11. Far AE, Aghakhani A, Hamkar R, Ramezani A, Pishbigar HF, Mirmomen S, Roshan MR, Vahidi S, Shahnazi V, Deljoodokht Z. Frequency of human papillomavirus infection in oesophageal squamous cell carcinoma in Iranian patients. Scand J Infect Dis 2007;39(1):58-62..
12. Moberg M, Gustavsson I, Gyllensten U. Real-time PCR-based system for simultaneous quantification of human papillomavirus types associated with high risk of cervical cancer. J Clin Microbiol 2003;41(7):3221-8.
13. Shahsiah R, Khademalhosseini M, Mehrdad N, Ramezani F, Nadji SA. Human papillomavirus genotypes in Iranian patients with cervical cancer. Pathol Res Pract 2011;207(12):754-7.
14. Evans MF, Adamson CS, Simmons-Arnold L, Cooper K. Touchdown General Primer (GP5+/GP6+) PCR and optimized sample DNA concentration support the sensitive detection of human papillomavirus. BMC Clin Pathol 2005;5:10.
15. Castillo A, Aguayo F, Koriyama C, Torres M, Carrascal E, Corvalan A, Roblero JP, Naquira C, Palma M, Backhouse C, Argandona J,Itoh T, Shuyama K, Eizuru Y, Akiba S. Human papillomavirus in esophageal squamous cell carcinoma in Colombia and Chile. World J Gastroenterol 2006;12(38):6188-92.
16. Shuyama K, Castillo A, Aguayo F, Sun Q, Khan N, Koriyama C, Akiba S. Human papillomavirus in high- and low-risk areas of oesophageal squamous cell carcinoma in China. Br J Cancer 2007;96(10):1554-9.
17. Souto Damin AP, Guedes Frazzon AP, de Carvalho Damin D, Beck Biehl H, Abruzzi de Oliveira L, Auler R, Marroni C, Alexandre CO. Detection of human papillomavirus DNA in squamous cell carcinoma of the esophagus by auto□nested PCR. Dis Esophagus 2006;19(2):64-8.
18. Takahashi A, Ogoshi S, Ono H, Ishikawa T, Toki T, Ohmori N, Iwasa M, Iwasa Y, Furihata M, Ohtsuki Y. High-risk human papillomavirus infection and overexpression of p53 protein in squamous cell carcinoma of the esophagus from Japan. Dis Esophagus 1998;11(3):162-7..
19. Kamath AM, Wu TT, Heitmiller R, Daniel R, Shah KV. Investigation of the association of esophageal carcinoma with human papillomaviruses. Dis Esophagus 2000;13(2):122-4.
20. Koshiol J, Wei WQ, Kreimer AR, Chen W, Gravitt P, Ren JS, Abnet CC, Wang JB, Kamangar F, Lin DM, von Knebel-Doeberitz M, Zhang Y, Viscidi R, Wang GQ, Gillison ML, Roth MJ, Dong ZW, Kim E, Taylor PR, Qiao YL, Dawsey SM. No role for human papillomavirus in esophageal squamous cell carcinoma in China. Int J Cancer 2010;127(1):93-100.
21. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005;32 Suppl 1:S16-24.
22. Molijn A, Kleter B, Quint W, van Doorn LJ. Molecular diagnosis of human papillomavirus (HPV) infections. J Clin Virol 2005;32 Supple 1:43-51.
23. Zehbe I, Voglino G, Wilander E, Genta F, Tommasino M. Codon 72 polymorphism of p53 and its association with cervical cancer. The Lancet 1999;354(9174):218-9.
24. Rys PN, Persing DH. Preventing false positives: quantitative evaluation of three protocols for inactivation of polymerase chain reaction amplification products. J clin microbiol 1993;31(9):2356-60.