Original Article

Prevalence of Human Papilloma Virus in Head and Neck Cancer in Thai Population

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Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is the sixth major cause of cancer morbidity and mortality in Thailand. The Human papilloma virus (HPV) has the important role in the pathogenesis of cervical cancer and sufficient evidence supports its role in carcinogenesis of HNSCC.

Objective: To investigate the presence of HPV infection in HNSCC in the Thai population and also further characterize the subtypes associated with the infected carcinoma.

Materials and Methods: Newly diagnosed HNSCC patients were recruited at the Division of Head Neck and Breast Surgery, Department of Surgery, Faculty of Medicine, Siriraj Hospital (Bangkok, Thailand). Fresh tissue samples were obtained for DNA preparation and the HPV DNA was detected by polymerase chain reaction.

Results: One hundred and thirty fresh tissue samples were obtained. Only one sample was positive for HPV type 16 DNA. This patient was a 56-years-old male patient with recurrent right palatine tonsil squamous cell carcinoma.

Conclusion: HPV infection is not the significant cause of HNSCC in Thai patients. Smoking, alcohol consumption, and betel nuts usage remain the major risk factor for HNSCC in the Thai population.

Keywords: Carcinogenesis, Cervical cancer, Head and Neck Squamous Cell Carcinoma, Human papillomavirus, Pathogenesis

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Head and Neck Squamous Cell Carcinoma (HNSCC), including cancer in oral cavity and oropharyngeal carcinoma, is a locally aggressive disease, resulting in a major cause of morbidity and mortality worldwide, with more than 800,000 new cases reported in 2018[1]. The incidence of this cancer is tending to increase among the young that are not prone to risk factors such as smoking and alcohol consumption[2]. HNSCC is the sixth[3] and tends to increase in rate every year though there are plenty of anti-smoking and anti-alcohol-drinking campaigns[4].

Human papillomavirus (HPV) is a family of icosahedral, non-enveloped viruses with a circular, double-stranded DNA genome of 7,500 to 8,000 base pairs (bp) and with a special affinity for epithelial cells[5,6]. HPV is well known to play an important role in the pathogenesis of cervical cancer worldwide in women[7,8]. Currently, many studies show sufficient evidence to conclude that HPV plays a role in the carcinogenesis of head and neck squamous cell carcinoma (HNSCC). Two of meta-analysis studies showed the high prevalence of HPV head and neck cancer[9,10]. Approximately 50% of oropharyngeal and tonsillar carcinomas are positive for HPV DNA, especially HPV type 16 has been identified in approximately 90% of HPV-infected tumors[11]. The high-risk type, HPV16, 18, 31, 33 are the main etiologic agents causing cancer of normal mucosa[9,12]. Smoking, alcohol consumption and betel nuts chewing are the well-known risk factors of HNSCC. The prevalence of the disease mostly occurs in the elderly and in men more than women. However, at present, higher prevalence of HNSCC are found in younger patients than in the past.

E6 and E7 transforming oncoproteins produced by HPV are capable of transforming normal cells into cancer cells by inactivating human tumor-suppressor proteins, p53 and retinoblastoma protein group (pRb), respectively. E6 and E7 genes are necessary for viral transformation and stimulate cellular proliferation, delay cellular differentiation, increase the frequency of spontaneous and mutagen-induced mutation and induce chromosomal instability in infected cell lines causing cellular immortalization[7,8,13].

Diagnosis of HPV is based on the use of molecular techniques. These methods can involve direct hybridization with DNA probes, such as Southern blotting or in situ hybridization; signal amplification, such as the hybrid capture method; or target nucleic acid amplification by the polymerase chain reaction (PCR) which is considered the most sensitive method for detecting HPV DNA[5,9,14,15].

HNSCC is one of the most malignant diseases in

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the Thai population tending to increase every year, especially in the young patients who are rarely expose to known risk factors. The aim of this study is first to investigate the presence of HPV DNA in head and neck cancer in the Thai population and also further characterize the subtypes associated with the HPV infected carcinoma.

Materials and Methods

Patients

Fresh tissue samples were obtained from newly diagnosed Thai HNSCC patients attending the Division of Head Neck and Breast Surgery, Department of Surgery, Faculty of Medicine, Siriraj Hospital (Bangkok, Thailand). The patients who were able to give informed consent were recruited. These samples were pathologically approved by two or more pathologists from the Department of Pathology, Faculty of Medicine, Siriraj Hospital. All consecutive samples were collected between November 2002 and April 2006. This study was approved by Siriraj Ethical Committee (COA No. Si176/2003).

DNA extraction

DNA extraction from fresh tissue was performed according to the methods described by Farhadi et al[16]. HeLa cell line[17] was used as a positive control. The samples were digested using lysis buffer containing 10 mmol/L Tris-HCl (pH 8), 100 mmol/L NaCl, 1% Sodium docecyl sulfate, 200 µg/ml proteinase K, and 0.01% EDTA at 56°C for 4 hours and then incubated overnight at 37°C. After digestion, proteinase K is inactivated by incubation at 95°C for 8 to 10 minutes. After vigorous shaking in 150 µl of phenol, chloroform, and isoamylalcohol (25: 24: 1) and spun for 2 minutes at high speed, the supernatant phase is transferred to a new tube. The DNA was recovered by ethanol precipitation.

Polymerase chain reaction

PCR was performed to detect HPV DNA by using oligonucleotide primers, MY09/MY11, GP5/GP6, CP-I/CP-IIG consensus primers which were obtained from Sigma-Proligo[18] (Table 1). β-Globin gene was used as a control for PCR reaction (primer PC03/PC04). Type specific (TS) primers for HPV16, HPV18, HPV31 and HPV33 were used for identification of the HPV subtypes if the sample was positive for HPV DNA[18] (Table 2).

The final 25-µl PCR mixture contained 2-µl DNA sample (50 ng/µl), 10 x PCR buffer (Tris-HCl 200 mM, pH8.4; KCl 500 mM), 25 mM $MgCl_2$, 10 mM deoxynucleoside triphosphate, 10 µM of each primer and 0.5 U of Taq DNA polymerase. The standard PCR conditions were preheated at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing temperature for 1 minute and extension at 40°C for 2 minutes followed by an additional 10 minutes at 72°C. Sterile water replacing the DNA sample was used as the negative control and HeLa DNA was used as a positive control. The PCR products were analyzed by agarose gel electrophoresis containing ethidium bromide and visualized under ultraviolet illumination.

Results

One hundred and thirty fresh tissue samples were obtained. Among these were 65 men and 65 women, and the mean patient age was 60.4 (range 21 to 100). The primary

Table 1. Specifications of oligonucleotides used as primers for general HPV detection by PCR[17]

Primers	Sequence (5'- 3')	Target	Length (bp)
MY09	CGTCCMARRGGAWACTGATC	L1	452
MY11	GCMCAGGGWCATAAYAATGG		
GP5	TTTGTTACTGTGGTAGATAC	L1	155
GP6	ACTAAATGTCAAATAAAAAG		
CP-I	TTATCAWATGCCCAYTGTACCAT	E1	188
CP-IIG	ATGTTAATWSAGCCWCCAAAATT		
PC03	ACACAACTGTGTTCACTAGC	β-Globin	100

Table 2. Specifications of oligonucleotides used as primers for type specific HPV detection by PCR[17]

Primer	Sequences (5'-3')	Amplimer length (bp)
HPV16 Forward	GGTCGGTGGACCGGTCGATG	96
HPV16 Backward	GCAATGTAGGTGTATCTCCA	
HPV18 Forward	CCTTGGACGTAAATTTTTGG	115
HPV18 Backward	CACGCACACGCTTGGCAGGT	
HPV31 Forward	GGGATTGTTACAAAGCTACC	110
HPV31 Backward	CGCTTAGTAGACGTCGTCGC	
HPV33 Forward	CCACCACTGCTTCTTACCTC	114
HPV33 Backward	ACCATTTTCATCAAATGGGA	

sites of tumor were tongue (60 samples), lip (16 samples), gum (14 samples), buccal mucosa (13 samples), cervical lymph node (8 samples), mandible (5 samples), floor of mouth (5 samples), hard palate (4 samples), larynx (2 samples), hypopharynx (1 sample), soft palate (1 sample) and palatine tonsil (1 sample).

All DNA samples were positive for β-Globin by using the PC03/PC04 primer set. HeLa DNA was positive by using the MY09/MY11 set and the CP-I/CP-IIG set, but negative by using the GP05/GP06 set. Only one sample was positive for HPV DNA by using TS primers for HPV16, but negative for other primer sets (Figure 1).

The only positive sample was obtained from a 56-year-old male patient with recurrent right palatine tonsil squamous cell carcinoma, rT2N0M0 (initial T2N1M0), clinical stage II. This patient has no history of smoking, alcohol consumption, betel nuts chewing, and family history of cancer. He died with disease 13 months after diagnosis.

Discussion

The well-known oncogenesis of head and neck cancer is caused by cigarette smoking and alcohol consumption[11,19]. Many studies showed the association between viral infection, especially HPV and EBV, and head and neck cancer[20]. One meta-analysis study showed the significant probability of HPV detection in oral cancer[9].

However, only one palatine tonsillar carcinoma sample was positive for HPV DNA in this present work. To ascertain the results, we chose the PCR assay, which is considered the most sensitive procedure for detecting HPV DNA[5,9]. Furthermore, this positive sample was repeatedly positive to only the TS primer for HPV16 in every experiment. The chosen TS primers designed by Baay et al were proved to be more sensitive for detecting HPV DNA in formaldehyde-fixed tissue samples than general primer pairs, GP05/GP06, MY09/MY11 and CP-I/CP-IIG[18]. Many studies reported HPV prevalence well over 90% amplifying the fresh tissue sample DNA by 100-bp amplimers in PCR[21-24].

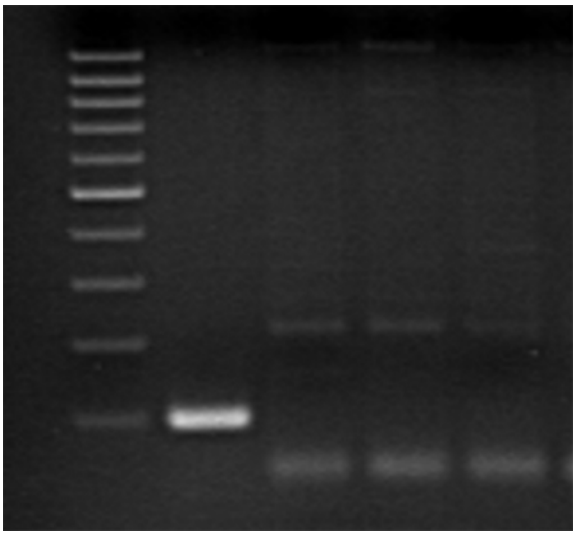

Figure 1. Gel electrophoresis of the PCR products using TS HPV primers. Lane 1: 100-base pair DNA ladder, Lane 2: PCR product by HPV16 primers, Lane 3: PCR product by HPV18 primers, Lane 4: product by HPV31 primers; Lane 5: product by HPV33 primers.

An absence of HPV in head and neck cancer using consensus primers has been reported[5]. The low prevalence of HPV detection has been reported as well[6,25-27]. Among these studies, tonsillar carcinoma specimens were mostly detected the HPV DNA. Recently, HPV16 is by far most frequent viral type involved in tonsillar carcinoma[26,28-31]. Two considerations in the process of viral oncogenesis have been suggested; site specificity and a shorter latent period for promotion of malignancy clones[25]. One suggested that, like the uterine cervix, the tonsils are located at the junction between the external and internal organs and are capable of metaplastic changes[32]. Besides, the tonsillar crypts may provide a large epithelial surface that may facilitate viral access to basal cells[28,33]. Alternatively, tonsillar epithelial cells may be uniquely sensitive to the transforming effects of the oncogenic virus[25,32-34].

Some authors believed that the role of chemical carcinogens seems to be more important in tumorigenesis than HPV infection[26]. The occasional HPV DNA detection in some head and neck cancer specimens may be resulted from the incidental HPV colonization rather than true infection[35].

Conclusion

Low prevalence of HPV DNA in the head and neck cancer of the Thai population suggested that this virus is not a significant cause of HNSCC in the Thai population. Other risk factors, such as smoking, alcohol consumption and betel nuts, may play major roles for developing this cancer among Thai patients.

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What is already known on this topic?

HNSCC is one of the most malignant diseases in Thai population tending to increase every year, especially in the young patients who rarely expose to known risk factors. The prevalence of the disease mostly occurs in the elderly and in men more than women. However, at present, higher prevalence of HNSCC are found in younger patients than in the past.

What this study adds?

HPV infection is not a significant cause of HNSCC in Thai patients. Other risk factors, such as smoking, alcohol consumption and betel nuts, may play major roles in developing this cancer among Thai patients.

Potential conflicts of interest

The authors declare no conflict of interest.

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อุบัติการณ์ของการติดเชื้อฮิวแมนแพพิโลมาไวรัสในผู้ป่วยมะเร็งศีรษะและลำคอชาวไทย

สรุจ ชัยศรีสวัสดิ์สุข, ดุลยพัฒน์ สงวนรักษา, ฐณวัฒน์ ธำรงธราดล, พรชัย โอเจริญรัตน์

ภูมิหลัง: มะเร็งศีรษะและลำคอชนิดสแควมัสพบได้เป็นลำดับที่ 6 ของผู้ป่วยมะเร็งทั้งหมดในประเทศไทย ฮิวแมนแพพิโลมาไวรัสมีบทบาทสำคัญในการเกิดมะเร็งปากมดลูก และมีหลักฐานสนับสนุนว่าการติดเชื้อนี้ส่งผลให้เกิดมะเร็งศีรษะและลำคอได้

วัตถุประสงค์: เพื่อศึกษาอุบัติการณ์การติดเชื้อฮิวแมนแพพิโลมาไวรัสและบอกสายพันธุ์ของไวรัสในผู้ป่วยมะเร็งศีรษะและลำคอชาวไทย

วัสดุและวิธีการ: การศึกษานี้รวบรวมผู้ป่วยรายใหม่ที่ได้รับการวินิจฉัยเป็นมะเร็งศีรษะและลำคอที่เข้ารับการรักษาที่สาขาศัลยศาสตร์ศีรษะ คอ และเต้านม ภาควิชาศัลยศาสตร์ คณะแพทยศาสตร์ศิริราชพยาบาล โดยทำการสกัดสารพันธุกรรมจากชิ้นเนื้อมะเร็งแล้วนำไปตรวจหาสารพันธุกรรมของฮิวแมนแพพิโลมาไวรัส

ผลการศึกษา: ในจำนวนผู้ป่วยทั้งหมด 130 ราย มีเพียง 1 ราย ที่มีการติดเชื้อฮิวแมนแพพิโลมาไวรัส โดยเป็นสายพันธุ์ 16 ผู้ป่วยรายนี้เป็นชายอายุ 56 ปี เป็นมะเร็งกลับเป็นซ้ำที่บริเวณทอนซิล

สรุป: การติดเชื้อฮิวแมนแพพิโลมาไวรัสไม่ใช่สาเหตุสำคัญของการเกิดมะเร็งศีรษะและลำคอในผู้ป่วยชาวไทย โดยสาเหตุหลักยังคงเป็นการสูบบุหรี่ การดื่มเครื่องดื่มแอลกอฮอล์ และการเคี้ยวหมาก