RESEARCH ARTICLE

Human Papillomavirus Genotypes and Cervical Cancer in Northeast Thailand

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Abstract

Human papillomavirus (HPV) is a major cause of cervical cancer. More than 100 HPV genotypes have been identified; however the distribution varies geographically and according to ethnicity. The purpose of this study was to investigate the prevalence and distribution of HPV subtypes among Northeast Thai women. Subjects included 198 cases of SCCA and 198 age-matched, healthy controls. HPV-DNA was amplified by PCR using the consensus primers GP5+/6+ system followed by reverse line blot hybridization genotyping. The prevalence of high-risk HPV infection was 21 (10.1%) and 152 (76.8%) in the controls and in the cases, respectively. High-risk HPV significantly increased the risk for cervical cancer with an OR of 42.4 (95%CI: 22.4-81.4, p<0.001) and an adjusted OR of 40.7-fold (95%CI: 21.5-76.8, p <0.001). HPV-16 was the most prevalent HPV type in the SCCA (56.2%) followed by HPV-58 (17.8%) and HPV-18 (13.6%); whereas HPV-58 (46.4%) was a prominent genotype in the controls followed by HPV-16 (39.3%) and unidentified HPV types (25.0%). These findings indicate that HPV infection remains a critical risk factor for SCCA; particularly, HPV-16, HPV-58 and HPV-18. In order to eradicate cervical cancer, sustained health education, promoted use of prophylactics and a HPV-58 vaccine should be introduced in this region.

Keywords: Cervical cancer - HPV genotype - HPV prevalence - Northeast Thailand

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Introduction

Cervical cancer is still a serious public health problem worldwide with an overall incidence of 15.2 per 100,000 (Ferlay et al., 2010) and a leading cause of death (14.4%) among female cancers in Thailand (National Cancer Institute, 2012).

Human papillomavirus (HPV) infection is a main cause for the development of cervical cancer (Liaw et al., 2001; Huang et al., 2004; Bhatla et al., 2008; Serrano et al., 2012). At present, more than 100 HPV genotypes have been identified (Huang et al., 2004; Kawana et al., 2012); among which 40 different types infecting the genital tract (Suthipintawong et al., 2011) are classified as high-risk type (HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -66 and -68) and associated with cervical carcinoma vs. the low-risk type (HPV-6, -11, -40, -42, -43, -44, -54, -61 and -72) associated with genital warts (Villa, 2006; Kawana et al., 2012).

Since the prevalence of HPV infection and distribution of HPV genotype varies with geographic area, ethnic background and life-style (Suthipintawong et al., 2011), knowing the distribution of HPV genotypes in a given area is important for public health decision-making. The current study investigated the prevalence of HPV infection and genotype distribution among Northeast Thai women vis-à-vis cervical carcinoma.

Materials and Methods

Study subjects

Women between 26 and 81years were recruited at Khon Kaen General Hospital and Srinagarind (University) Hospital, Khon Kaen Province, Northeast Thailand, between February 2009 and August 2011. There were 198 cases with pathology-defined squamous cell carcinoma of the cervix (SCCA) and 198 age-matched healthy controls with normal cytology (Pap smear) and histology.

This study was approved by the Ethics Committee of Khon Kaen University (HE 450333) and Khon Kaen Hospital (No. 03/02/2554).

Cervical cell collection

Cervical cells were collected by Pap smear and the remaining cells suspended in 10 ml of phosphate buffered saline (PBS) were used in this study. Cell suspension was centrifuged at 3,500 rpm for 10 min and cell pellet was washed with 900 μl of PBS and centrifuged at 3,500 rpm

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for 10 min. Collected cells were kept at -20°C prior to HPV detection and typing.

HPV detection and typing

The DNAs of the cervical cells were extracted using Genomic DNA (blood/cells) Mini Kit (Geneaid, Taiwan). PCR amplification for HPV DNA was performed using the GP5+/GP6+ consensus primers for the conserved region in the L1 open reading frame of the HPV genome as previously described (Camargo et al., 2011). Primer GP6+ was biotinylated to yield labeled PCR products. The biotin labeled PCR products of each sample were genotyped using reverse line blot hybridization (RLBH) (van den Brule et al., 2002). Biodyne C blotting membrane (Gelman Science Ins, MI, USA) was activated in 16% (w/v) 1-ethy-3-(3-dimethylaminopropyl) carbodimide (EDAC) solution (Sigma-Aldrich, St. Louis, MO, USA) for 10 min at room temperature, rinsed with distilled water and placed on mini blotter. Thirty-seven HPV type-specific 5'-amino-linked oligonucleotides probes—including 13 high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68); 12 low-risk HPV types (6, 11, 26, 40, 42, 43, 44, 53, 54, 61, 72 and 73); and, other HPV types (34, 55, 57, 66, 70, 82MM4, 83MM7, 84MM8, 82IS39, CP6108, 71CP8061 and 81CP8304)—were dropped on Biodyne C membrane through the wells of the mini blotter in parallel lines. Subsequently, biotin-labeled PCR products were pipetted into the channels of the mini blotter perpendicular to the oligonucleotides probe lines, then hybridized and incubated with streptavidin-peroxidase-conjugate. The HPV types were detected using chemiluminescence.

To confirm the success of the DNA extraction, β-globin PCR was applied to HPV DNA-negative samples. The amplified products were verified on 2% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light.

Statistical analyses

The χ^2-test was used for the statistical examination. Association between HPV infection and risk for SCCA was studied using uni- and multi-variate logistic regression analyses (using 800-STATA on PC) (p value<0.05).

Results

The prevalence of HPV infection was 28 (14.1%) and 169 (85.4%) in the controls and cases, respectively. HPV infection significantly increased the risk for cervical cancer with an OR of 35.38 (95%CI: 19.50-64.62, p<0.001) and an adjusted OR of 34.84 (95%CI: 19.13-63.44, p<0.001). A variety of frequencies of low-risk, high-risk and unidentified types of HPV infection were observed (Table 1). High-risk HPV carriers numbered 21 and 152 in the controls and cases, respectively. Infection with the high-risk HPV significantly increased the risk for cervical cancer (40.67-fold; 95%CI: 21.53-76.81, p<0.001) after adjustment for number of sexual partners, age at first intercourse, age at first delivery, number of pregnancies, number of parities, oral contraceptive pills use and injection contraceptive use. An increased OR and adjusted OR were also found among the unidentified types of HPV: 10.05 (95%CI: 3.28-32.30, p<0.001) and 10.95 (95%CI: 3.65-32.84, p<0.001), respectively.

The distribution of HPV genotypes among Northeast Thai women is presented in Table 2. Among the HPV carriers (n=28) in the controls, HPV58 was found in 13 followed by HPV-16 (n=11) and unidentified types of HPV (n=7). Among the 169 HPV carrying patients with SCCA, 95 harbored HPV-16 followed by HPV-58 (n=30) and HPV-18 (n=23). Several combinations of double-, triple- and quadruple-HPV infection were also detected in this population.

Discussion

The prevalence of HPV infection among Thai women without cervical abnormalities was between 5~15% (Chichareon et al., 1998; Sukvirach et al., 2003;

Table 2. Distribution of HPV Genotypes in Cervical Cancer among Northeast Thai Women

HPV genotypes	Cases, n	Controls, n	
Low-risk	HPV-11	1	0
	HPV-42	3	0
	HPV-72	1	0
	Unidentified	12	7
High-risk			
Single infection	HPV-16	86	4
	HPV-18	20	1
	HPV-31	0	0
	HPV-33	6	1
	HPV-39	2	0
	HPV-45	3	1
	HPV-52	2	0
	HPV-58	22	5
	HPV-59	0	0
Double infection	HPV-16/18	1	1
	HPV-16/52	1	0
	HPV-16/58	4	2
	HPV-16/59	1	0
	HPV-18/58	0	1
	HPV-33/58	2	1
Triple infection	HPV-16/18/58	1	2
Quadruple infection	HPV-16/18/31/58	1	0
	HPV-16/18/33/58	0	2
Total		169	28

Table 1. Prevalence of HPVInfection among Northeast Thai Women

HPV status	Cases, n (%)	Controls, n (%)	OR, (95%CI, p value)	Adjusted OR*, (95%CI, p value)
Negative	29 (14.65)	170 (85.86)	1	1
Positive	169 (85.35)	28 (14.14)	35.38 (19.50-64.62, <0.001)	34.84(19.13-63.44, <0.001)
Low-risk types	5 (2.53)	0 (0.00)	**	**
High-risk types	152 (76.77)	21 (10.10)	42.43 (22.35-81.38,<0.001)	40.67 (21.53-76.81, <0.001)
Unidentified types	12 (6.06)	7 (3.54)	10.05 (3.28- 32.30, <0.001)	10.95 (3.65-32.84, <0.001)

*Adjusted multiple logistic regression for number of sexual partners, age at first intercourse, age at first delivery, number of pregnancies, number of parities, oral contraceptive pills use and injection contraceptive use; **Drop because confidence levels not possible

Chansaenroj et al., 2010). The prevalence in Khon Kaen, northeastern Thailand was 13% in 2003 (Settheetham-Ishida et al., 2005). The present study confirms the persistence of HPV infection (14.14%) among women in Khon Kaen. When we investigated HPV infection among women with SCCA, the prevalence of HPV infection ranged between 83%~97% (Tungsinmunkong et al., 2006; Siriaunkgul et al., 2008; Chinchai et al., 2012). There was one report (Suthipintawong et al., 2011) using FFPE specimens collected between 2004 and 2007 and analyzed for HPV DNA using a standardized method. This study covered the four regions of Thailand and illustrated the prevalence HPV infection was 93.3% in the North, 100% in the Northeast, 80% in the Central region and 100% in the South, with an overall prevalence of HPV infection as 93.3%. In our study, the prevalence of HPV infection was 85.4% of the cases with an increased risk for development of SCCA (as high as 34.84-fold). Three-quarters (76.6%) of the cases were infected with high-risk HPV which increased the risk for SCCA as much as 40.67-fold. Our study thus confirmed that HPV infection remains a crucial cause of cervical cancer in this part of the world.

The detection of low risk HPV infection in SCCA (including three cases of HPV-42) should be into consideration because HPV-42 infection has been found in women with normal cytology in Thailand (Sukvirach et al., 2003; Lurchachaiwong et al., 2011) and can pose a potential risk for developing cervical cancer. Evaluation of HPV genotype distribution must be considered with the typing method used because we observed that HPV-42 turned to be common when GP5+/6+ primer set was used (de Sanjose et al., 2007).

In accord with several previous reports from Thailand (Chichareon et al., 1998; Siriaunkgul et al., 2008; Suthipintawong et al., 2011; Chinchai et al., 2012) and Asia (Huang et al., 2004; Bhatla et al., 2008; Chinchai et al., 2012; Kawana et al., 2012), we confirmed that in Northeast Thailand HPV-16 is the most prevalent type of HPV among women with SCCA (56.21%). In addition to the high prevalence of single infection of HPV-16 in SCCA (50.9%), co-infection with HPV-16 was common among those with multiple HPV infections (9 out of 11). The frequent presence of HPV-16 among multiple HPV infections might be attributed to its ubiquity but as much to its nature; in that a preexisting infection supports subsequent acquisition and persistence of other types of HPV infection (Liaw et al., 2001).

As for the prevalence of the various types of HPV, the general experience is the high prevalence of HPV-16 followed by HPV-18 (Chichareon et al., 1998; Settheetham-Ishida et al., 2005; Suthipintawong et al., 2011); however, our results indicated a rather high prevalence of HPV-58 (17.8%) in SCCA. HPV-58 is not rare in Asian women with normal cervical cytology; its prevalence being <1% in Thailand, Vietnam, Japan and Korea (Domingo et al., 2008; Konno et al., 2008) and 1~2% in Taiwan and China (Shi et al., 2008; Tay et al., 2008). Among SCCA patients, a condensation of HPV-58 infection (about 26%) was reported in Shanghai (Huang et al., 1997), whereas it reached 18% in Khon Kaen (present study; Sriamporn et al., 2006). An interesting association between HPV-58 infection in SCCA and HLA-DQB1*06 was reported (Chan et al., 2007). Certain HLA haplotypes alter disease susceptibilities vis-à-vis the relationships between HTLV-1 infection and ATL and/or HAM (Sonoda, 1990) and EBV and NPC (Goldsmith et al., 2002), which were well documented viral oncology issues. Genetic composition of the Thai population has Chinese contributions and HLA-DQB1*06 is common especially among northeastern Thais (Romphruk et al., 1999; Romphruk et al., 2010). If the HLA type has an increasing effect on SCCA development, a rather high prevalence of HPV-58 in SCCA patients in northeastern Thailand would be consistent.

At present, two prophylactic vaccines have been licensed for clinical use worldwide; the bivalent vaccine (Cervarix™) and the quadrivalent vaccine (Gardasil®). Both contain HPV-16 and -18 epitopes as the main components (Villa, 2006; Serrano et al., 2012). Since their prophylactic spectra are limited to HPV-16, -18 (and additionally -6 and -11 for Gardasil®), only 70% of invasive cervical cancer cases can be prevented worldwide (Serrano et al., 2012; Xue et al., 2012; Yue et al., 2013) and also in Thailand. Common dissemination of HPV-58 as well as -33 and -31 in Thailand (Sukvirach et al., 2003; Domingo et al., 2008) demands invention and circulation of a novel vaccine targeting these minor but burdensome HPV strains.

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