RESEARCH ARTICLE

Risk Factors for Cervical Cancer in Northeastern Thailand: Detailed Analyses of Sexual and Smoking Behavior

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Abstract

Cervical cancer is a serious public health problem in Thailand. We investigated possible risk factors for cervical cancer including HPV infection, *p53* polymorphism, smoking and reproductive history among women in Northeast Thailand using a case control study with 177 cases and age-matched controls. Among the HPV carriers, a significantly increased risk for cervical cancer with an OR of 36.97 (p<0.001) and an adjusted OR of 38.07 (p<0.001) were observed. Early age at first sexual exposure, and multiple sexual partners increased the risk of cervical cancer with ORs ranging between 1.73-2.78 (p<0.05). The interval between menarche and first sexual intercourse <6 years resulted in a significant increase in the risk for cervical cancer with ORs ranging between 3.32-4.09 and the respective adjusted OR range for the 4-5 and 2-3 year-old groups were 4.09 and 2.92. A higher risk was observed among subjects whose partner had smoking habits, whether currently or formerly; with respective ORs of 3.36 (p<0.001) and 2.17 (p<0.05); and respective adjusted ORs of 2.90 (p<0.05) and 3.55 (p<0.05). Other smoking characteristics of the partners including smoking duration ≥ 20 years, number of cigarettes smokes ≥ 20 pack-years and exposure time of the subject to passive smoking ≥ 5 hrs per day were found to be statistically significant risks for cervical cancer with adjusted ORs of 3.75, 4.04 and 11.8, respectively. Our data suggest that the risk of cervical cancer in Thai women is substantially associated with smoking characteristics of the partner(s), the interval between menarche and first sexual intercourse as well as some other aspects of sexual behavior.

Keywords: Menarche - sexual exposure - HPV infection - smoking - cervical cancer - Northeast Thailand

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Introduction

Cancer of the cervix is one of the most serious public health problems among Thai women (National Cancer Institute et al., 2010). It is now widely accepted that high risk types of human papillomavirus (HPV) (Moscicki et al., 2001; Klug et al., 2009; Carter et al., 2011), particularly HPV 16 and 18, play an important role in the genesis of cervical carcinoma (zur Hausen, 1991). Most HPV infections in the cervix spontaneously resolve and few (not all) HPV-infected females develop cervical cancer (Josefsson et al., 2000; Nagpal et al., 2002). Other risk factors such as exposure to certain carcinogens and host genetic predisposition likely have an influence on cervical carcinogenesis.

TP53 is a tumor suppressor protein with a highly conserved role as a 'guardian of the genome' via cellular anticancer mechanisms (Ji et al., 2008). A base substitution at codon 72 of exon 4 of gene *p53* that results in either arginine (Arg; CGC) or proline (Pro; CCC) has been identified as polymorphic in human populations (Matlashewski et al., 1987; Dybikowska et al., 2000; Comar et al., 2004; Klug et al., 2009). The association of the *p53* codon 72 polymorphism with an increased susceptibility for development of cancer has been examined for many sites (*e.g.*, lung cancer (Piao et al., 2011), gastric cancer (Perez-Perez et al., 2005), and endometrial cancer (Roh et al., 2004)). The HPV E6 oncogene protein binds to TP53 and promotes dysfunction of its activity. Storey et al. (1988) suggest that homozygosity for the Arg allele was more marked with overrepresentation than the presence of the Pro allele, in HPV-associated cancers (Storey et al., 1998). The effect of the *p53* polymorphism on cervical cancer may therefore be related to cervical carcinogenesis.

Tobacco smoke contains more than 4,000 chemical substances including some carcinogens (Lodovici et al., 2009). Among these, polycyclic aromatic hydrocarbons (PAHs) and volatile N-nitrosamines are considered to be the main carcinogens (Shields, 2002; Alam et al., 2008; Lodovici and Bigagli, 2009). Smoking exposure-a well-documented environmental factor-is a leading cause of

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many types of cancer such as lung, esophageal, gastric, bladder, liver and cervical cancers (Tredaniel et al., 1997; Kinjo et al., 1998; Gallus et al., 2001; Sobue et al., 2002 ; Settheetham-Ishida et al., 2004; Samanic et al., 2006; Settheetham-Ishida et al., 2006; Syrjanen et al., 2007; Pesch et al., 2011). An increased risk of cervical cancer associated with tobacco smoking has been found in many studies (Haverkos et al., 2003; Settheetham-Ishida et al., 2004; Garland et al., 2011; Yetimalar et al., 2011). Among HPV-positive women, an increased risk of cervical cancer was demonstrated among smokers than non-smokers (Kapeu et al., 2009; Plummer et al., 2003). There are, however, some conflicting data with regards to the association between smoking and cervical cancer in southern European populations (Matos et al., 2005). These contradictory results could be attributed to differences in the characteristics of the smoking habit (*i.e.*, the amount of tobacco smoked and the number of years as a smoker). Indeed, the possible association between passive smoking and cervical carcinogenesis has not been thoroughly evaluated.

There are therefore several risk factors for cervical cancer (*viz.*, HPV infection, sexual behaviors, *p53* polymorphism and the characteristics of smoking) and these will in turn be related to tumorigenic potential (Hsieh et al., 2005). The purpose of our study was to examine the risk of cervical cancer with special respect to sexual behavior and tobacco smoking habits (whether active or passive).

Materials and Methods

Women between 27 and 81 years of age were recruited between February 2009 and August 2011 at Khon Kaen Hospital and Srinagarind Hospital, Khon Kaen Province, in Northeastern Thailand. The study comprised 177 cases and 177 controls. The cases had a confirmed diagnosis of squamous cell carcinoma of the cervix (SCCA), by pathological examination. Controls were recruited among healthy woman with normal cytology (Pap smear) and histology.

The controls and cases were matched within 5-year age groupings. The subjects were verbally informed and received documentation explaining the purposes and procedures involved in the study. All of the subjects signed an informed consent form prior to participation in the study. The variables of interest were obtained through direct interview, including: behavioral data (age at menarche, number of sexual partners, age at first intercourse, age at first delivery, number of pregnancies, number of parities, and contraceptives use). Information regarding the details of smoking of both the subjects and their partners were recorded (*i.e.*, smoking status, smoking duration, pack-years and smoking exposure). This study was reviewed and approved by the Ethics Committee of both Khon Kaen University (HE 450333) and Khon Kaen Hospital (No. 03/02/2554).

Detection of p53 codon 72 polymorphism

Genomic DNA was extracted from the buffy coat using GF-1 Blood DNA Extraction Kits (Vivantis, USA). The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was used for analysis for *p53* codon 72 polymorphism (Kietthubthew et al., 2003). PCR was then performed with the following primers to amplify the *p53* exon 4: 5'-CCCGGACGATATTGAACA-3' and 5'-AGAAGCCCAGACGGAAAC-3'.

The PCR products of 203 base pairs (bp) were loaded into 2% agarose gel. After electrophoresis, the gels were stained with ethidium bromide and photographed under UV light. The PCR product was digested using *BstU*I (New England, USA). The *BstU*I recognizing the CGCG sequence in the Arg allele generated a 125 bp and a 78 bp fragment, whereas the CCC or Pro allele remained uncut.

Detection of HPV

The DNA from cervical cells was extracted using Genomic DNA (blood/cells) Mini Kit (Geneaid, Taiwan). The samples were tested for the presence of HPV DNA using PCR amplification of the L1 region with the GP5+/GP6+ consensus primers (GP5+ 5'-TTTGTTACTGTGGTAGATACTAC-3' and GP6+ 5'-GAAAAATAAACTGTAAATCATATTC-3') (Camargo et al., 2011). The amplified product was verified by 2% agarose gel electrophoresis, stained with ethidium bromide and visualized under UV light. Beta-globin was used as the internal control.

Statistical analyses

The genotypic frequencies between the case and controls were compared using the χ^2-test. The association between selected variables and risk for SCCA were studied using uni- and multi-variate logistic regression analyses (using 800-STATA on PC) and the odds ratio (OR) at the 95% confidence interval (CI) was calculated. Differences were considered statistically significant when the p-value was <0.05.

Results

The distributions of variables selected as potential risk factors for cervical cancer in the cases and controls are summarized in Table 1. The genotype distribution of the *p53* polymorphism met the Hardy-Weinberg equilibrium in the cases and controls, the respective distributions of the alleles between the cases and controls were not significantly different ($p>0.05$). The genotype distribution of *p53* polymorphism did not significantly alter the risk for SCCA ($p>0.05$). Significant differences were, however, observed for HPV infection, smoking exposure, age at first delivery (≤18 years), age at first sexual intercourse (≤16 years), prolonged use of injective contraception (>2 years), prolonged use oral contraceptive pills (>2 years), multiple sexual partners (>1), multiple pregnancies (≥3) and multiple parities (≥3). Prevalence of HPV infection among patients diagnosed with cervical cancer (case group) was 85.9% *vs*. 14.1% in the control group. After adjusting with multiple logistic regression, increased risk for SCCA persisted for HPV infection and smoking exposure with a respective adjusted OR of 38.07 ($p<0.001$) and 3.01 ($p=0.001$).

Table 1. Selected Risk Factors for Cervical Cancer

Variables		Cases n	Controls n	OR [95% CI, p-value]	Adjusted OR[a] [95% CI, p-value]
p53 polymorphism					
Pro/Pro		39	52	1	1
Arg/Arg		57	49	1.55 [0.85-2.84, 0.1265]	1.98 [0.83-4.70, 0.124]
Arg/Pro		81	76	1.42 [0.82-2.47, 0.1846]	1.46 [0.66-3.23, 0.354]
Arg/Arg+Arg/Pro		138	125	1.47 [0.89-2.45, 0.1139]	1.64 [0.78-3.45, 0.188]
Allele distribution[b]	Pro	0.45	0.51	NA	NA
	Arg	0.55	0.49	NA	NA
HPV status	Negative	25	152	1	1
	Positive	152	25	36.97 [19.56-70.45, <0.001]	38.07 [19.67-73.66, <0.001]
Age at menarche	> 12 years	165	168	1	1
	≤ 12 years	12	9	1.36 [0.51-3.75, 0.4997]	1.47 [0.42-5.16, 0.549]
Number of sexual partners	≤ 1	126	147	1	1
	> 1	51	30	1.98 [1.16-3.43, 0.0079]	1.45[0.67-3.11, 0.342]
Age at first intercourse	> 16 years	155	167	1	1
	≤ 16 years	22	10	2.37 [1.03-5.78, 0.0261]	0.87[0.24-3.20, 0.831]
Age at first delivery	> 18 years	135	158	1	1
	≤ 18 years	42	19	2.59 [1.39-4.94, 0.0012]	2.12 [0.75-6.01, 0.158]
Number of pregnancies	< 3	56	81	1	1
	≥ 3	121	96	1.82 [1.16-2.88, 0.0064]	1.14 [0.42-3.09, 0.796]
Number of parities	< 3	83	107	1	1
	≥ 3	94	70	1.73 [1.11-2.70, 0.0105]	1.63 [0.62-4.28, 0.325]
Oral contraceptive pill use	< 2 years	114	140	1	1
	≥ 2 years	63	37	2.09 [1.27-3.47, 0.0021]	1.45 [0.70-3.04, 0.317]
Injection contraceptive use	< 2 years	147	162	1	1
	≥ 2 years	30	15	2.20 [1.10-4.58, 0.0167]	1.71 [0.64-4.53, 0.283]
Smoking exposure[c]	No	52	95	1	1
	Yes	125	82	2.78 [1.76-4.42, <0.001]	3.01 [1.54-5.88, 0.001]

[a]djusted multiple logistic regression. [b]in frequency. [c]Yes denotes that the subject or her partner(s) had smoking habit; No denotes that both were non smokers. NA: not applicable

Table 2. Interval Between Menarche and First Sexual Intercourse and Risk for Cervical Cancer

Interval (year)	Cases n	Controls n	OR [95% CI, p-value]	Adjusted OR[a] [95% CI, p-value]
≥ 6	44	96	1	1
4-5	38	25	3.32 [1.71-6.46, <0.001]	4.09 [1.61-10.42, 0.003]
2-3	44	26	3.69 [1.94-7.06, <0.001]	2.92 [1.12-7.61, 0.028]
0-1	45	24	4.09 [2.13-7.91, <0.001]	2.05 [0.74-5.64, 0.166]

[a]adjusted multiple logistic regression for HPV infection, 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

Table 3. Partners' Smoking Habits and Risk for Cervical Cancer

	Variables	Case n	Control n	OR [95%CI, p-value]	Adjusted OR[a] [95%CI, p-value]
Smoking status	Non smoker	53	96	1	1
	Smoker	124	81	2.77 [1.75-4.40, <0.001]	3.15 [1.62-6.13, 0.001]
	Current smoker	76	41	3.36 [1.96-5.76, <0.001]	2.90 [1.37-6.14, 0.005]
	Former smoker	48	40	2.17 [1.23-3.85, 0.0043]	3.55 [1.55-8.16, 0.003]
Smoking duration (year)	0	51	97	1	1
	<20	43	31	2.64 [1.43-4.88, 0.0008]	3.31 [1.37-8.01, 0.008]
	≥20	83	49	3.22 [1.92-5.42, <0.001]	3.75 [1.78-7.91, 0.001]
Pack-years	0	46	89	1	1
	≤9	60	32	3.63 [2.00-6.59, <0.001]	4.28 [1.83-10.02, 0.001]
	10-19	34	28	2.35 [1.21-4.55, 0.0059]	1.92 [0.78-4.69, 0.155]
	≥20	37	28	2.56 [1.33-4.91, 0.0021]	4.04 [1.56-10.44, 0.004]
Smoking exposure (hr/day)	0	52	93	1	1
	<5	108	79	2.44 [1.53-3.92, 0.0001]	2.71 [1.37-5.36, 0.004]
	≥5	17	5	6.08 [1.98-22.08, 0.0002]	11.76 [2.93-47.16, 0.001]

[a]adjusted multiple logistic regression for HPV infection, 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 Pack-years is calculated by multiplying the number of packs of cigarettes smoked per day by the number of years the person has smoke

The current study also demonstrated that women who had sexual intercourse within 5 years of menarche had a significantly increased risk of SCCA (p<0.001) with ORs ranging between 3.32-4.09 compared with women who postponed first sexual intercourse 6 years beyond menarche (Table 2). After adjusting the multiple logistic regression, however, a significantly increased risk for SCCA was found in those engaging in sex 4-5 and 2-3 years after menarche with a respective adjusted OR of 4.09 and 2.92.

Table 4. Combination of Smoking Duration, Pack-years and Smoking Exposure and Risk for Cervical Cancer

dpe[b]	Case n	Control n	OR [95%CI, p-value]	Adjusted ORa [95%CI, p-value]
0	48	89	1	1
>0-≤100	31	21	2.74 [1.35-5.57, 0.0022]	5.55 [2.00-15.36, 0.001]
>100-≤500	47	24	3.63 [1.90-6.97, <0.0001]	3.33 [1.38-8.04, 0.008]
>500-≤1000	16	18	1.65 [0.71-3.77, 0.1948]	3.38 [1.05-10.84, 0.040]
>1000	24	14	3.18 [1.42-7.26, 0.0018]	4.68 [1.51-14.46, 0.007]

[a]adjusted multiple logistic regression for HPV infection, 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, [b]dpe is calculated by multiplying the smoking duration by number of packs years and smoking exposure time (smoking duration x pack-years x smoking exposure)

The smoking habits of a partner are presented in Table 3. There was an increased risk for SCCA (OR = 2.77 [p<0.001] and adjusted OR = 3.15 [p=0.001]) in females with sexual partners who were smokers. A higher risk for cervical cancer was detected in both current and former smokers with a respective OR of 3.36 (p<0.001) and 2.17 (p<0.01) and a respective adjusted OR of 2.90 (p<0.01) and 3.55 (p<0.01). Long-term smokers (*i.e.*, 20 or more years) and amount of tobacco smoked (*i.e.*, 20 or more pack-years) by partners as well as exposure to smoking (5 or more hr/day) demonstrated a statistically significant risk for SCCA with a p-value <0.01 with respective adjusted ORs of 3.75, 4.04 and 11.76. The combination of a partner's smoking habits (duration and amount) and exposure resulted in a dpe value of more than 1000, which indicates a significant risk for SCCA with an odds ratio of 3.18 (p<0.01) and an adjusted odd ratio of 4.68 (p<0.01) (Table 4).

Discussion

The current study, strongly confirmed that HPV infection remains a crucial cause of SCCA with an adjusted OR of 38.07 (p< 0.001). The prevalence of HPV infection among patients (85.9%) and controls (14.1%) seems higher compared to the previous study reporting the positivity rate 12% and 78% for the patients and controls, respectively. This may be attributable to the detection of only high risk HPV infections in the latter study (Settheetham-Ishida et al., 2005), a further HPV-typing will clear the gap of the prevalence. HPV infection was also detected in the controls indicated that not all of the HPV-infected women developed cervical cancer. Among healthy women, an HPV infection was able to clear within 1–2 years (Klug et al., 2009; Schiffman et al., 2007) and <1% of HPV-positive women would go on to develop cervical cancer (Josefsson et al., 2000; Nagpal et al., 2002). The current report indicates that the involvement of other factors such as sexual behavior, personal and partner smoking habits and genetic backgrounds are likely co-factors with HPV for an increased risk of developing cervical cancer (Settheetham-Ishida et al., 2004; Settheetham-Ishida et al., 2005; Au et al., 2007; Almonte et al., 2008).

The HPV co-factors may act with HPV via two or more possible mechanisms. First, co-factors may promote HPV infection by causing cervical epithelial injury, such as: early age at first sexual activity and first delivery, multiple pregnancies and/or multiple sexual partners (Kahn et al., 2002; Matos et al., 2005; Louie et al., 2009). Second, co-factors may promote persistent infection of HPV in cervical tissues, such as: prolonged use of contraceptive pills (Delvenne et al., 2007), leading to increased risk for cervical cancer in longer use of contraceptive in this study. Estrogen contained in oral contraceptives may affect cervical carcinogenesis in several ways, such as: increasing the S-phase fraction (Bhattacharya et al., 1997), increasing the sensitivity of cervical transformation zone (Hughes et al., 1988; Castellsague et al., 2003) and binding to a specific DNA sequences within transcriptional regulatory regions on the HPV DNA either to increase or to suppress transcription of various genes (Moodley et al., 2003). On the other hand, long-term consumption of oral contraceptives among women may reflect potentially frequent sexual activity, which may also increase the risk of contracting HPV or other sexually-transmitted diseases.

In the present study, the mean age at the first sexual intercourse was 20.85 years compared to 17.6-18.0 years in Northern Thailand (Allen et al., 2003; Liu et al., 2006), 13.0-16.2 in America (Zelnik et al., 1983; Coker et al., 1994; De Genna et al., 2011), 14.0-16.8 in Europe (Andersson-Ellstrom et al., 1996; Wellings et al., 1996; Edgardh, 2000; Woynarowska et al., 2006; Panatto et al., 2012) and 13.5-17.8 in Africa (Kayembe et al., 2008 ; Dingeta et al., 2012; Doku, 2012; Remes et al., 2012). The delay of first sexual intercourse in the current study may have been influenced by Thai traditional culture, in which early experience with sexual intercourse is considered socially unacceptable (Sridawruang et al., 2010; Supametaporn et al., 2010). Biologically, we found that early sexual activity after menarche (< 6 years) led to greater susceptibility to SCCA. During puberty, the immaturity of cervical cells undergo many changes that might be more vulnerable to damage and HPV infection and/or other sexually-transmitted diseases (Coker et al., 1994; Kahn et al., 2002; Collins et al., 2005; Matos et al., 2005; Louie et al., 2009). This data indicates that a delay of 6 or more years after menarche before sexual intercourse would be safer and afford some protection from sexually-transmitted diseases (including HPV infection) and susceptibility to cervical cancer.

Smoking is not generally socially acceptable among Thai women. The prevalence of tobacco smoking among Thai females is therefore only 4.84% of Thai smokers (National statistical office et al., 2012). Consequently, most women are exposed to tobacco smoking passively from sexual partners. Our study demonstrated that there was an increased risk for SCCA in Thai females who had partners designated as current smokers and former

smokers with ORs of 3.36 (p<0.001) and 2.17 (p<0.05), respectively. The current study has indicated that both current and former smoking status of the sexual partners increased the risk of SCCA. This trend confirms previous work in the region (Settheetham-Ishida et al., 2004) that passive tobacco smoking contributed to an increased risk of cervical cancer development with an adjusted OR of 3.15 [95%CI;1.62-6.13, p=0.001] among Northeast Thai women. Some components of tobacco smoke—such as nicotine, cotinine, and benzo(α)pyrene can be detected in the cervical mucus (Simons et al., 1993; Prokopczyk et al., 1997; Coker et al., 2002). The possible routes of the components get access into the uterine cervix by inhalation and/or semen exposure (Kulikauskas et al., 1985; Perrin et al., 2011). These carcinogens could affect HPV DNA replication as well as modulate the HPV life cycle potentially enhancing viral persistence leading to host tissue carcinogenesis (Castellsague and Munoz, 2003; Syrjanen et al., 2007). Moreover several substances commonly found in cigarette smoke may activate carcinogenic nitrosamines leading to DNA damage which may impair local defense mechanisms within the cervical epithelium (Kjellberg et al., 2000). Thus, women exposed to second-hand or passive tobacco smoke are at risk.

The characteristics of a partner's smoking habit were clarified in the current study. Duration and amount of tobacco smoking and smoking exposure of the subject can increase the risk for cervical cancer. The combination of smoking duration, pack-years and smoking exposure has a synergistic effect on cervical cancer development. To reduce the risk of smoking-related cancer, all of these factors need to be addressed.

In conclusion, HPV infection, reproductive behaviors and smoking are the main risk factors for cervical cancer among Thai females. Health education for a healthy lifestyle with a reduced risk of cervical cancer should include: appropriate timing for first sexual exposure, appropriate use of contraceptive methods and avoidance of passive smoking.

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